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VOLUME 26 NOVEMBER 1938 NUMBER 5

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CARBON TETRACHLORIDE POISONING

A STUDY OF STAGES OF HEPATIC DAMAGE AND REPAIR IN MAN

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WASHINGTON, D. C.

Carbon tetrachloride is a chemical agent widely used in industry. It is used [1] as a general solvent, in fire extinguishers, in cleaning compounds, in the manufacture of chloroform, in chlorinating organic compounds, as a substitute for oil in electrical transformers and high tension switches, as a solvent for fats and oils, in electroplating, in metal polishes, in lacquers, for extracting perfume from flowers, as a paraffin solvent, for recovering waxes and resins from raw materials, in rubberizing fabrics, in rubber cements, in the manufacture of gelatinous, water-soluble soaps from sulfonated oils and resins, and for degreasing textiles. Other known uses are: as a fumigant [2]; in milliner's cement [3]; in certain hair tonics [4]; in certain "dry shampoos" [5]; as a local anesthetic [6]; as an anthelmintic.

HUMAN POISONING WITH CARBON TETRACHLORIDE

That its use in industry is not devoid of danger has been pointed out repeatedly. The commonest form of the hazard is that of exposure to the fumes of the chemical. Following a single toxic exposure the clinical manifestations are usually headache, nausea, vomiting and mental confusion; exposure to concentrated fumes in a poorly ventilated room may result in deep anesthesia.[7] Later manifestations of acute intoxication or of repeated exposures are usually jaundice, suppression of urine and anemia.

From the Department of Pathology, Medical College of the State of South Carolina, Charleston, S. C.

1. Turner, F. M.: The Condensed Chemical Dictionary, ed. 2, New York, Chemical Catalog Company, Inc., 1930.

2. Haigler, F. H.: U. S. Nav. M. Bull. **30**:137, 1932.

3. Hamilton, A.: Industrial Poisons in the United States, New York, The Macmillan Company, 1929.

4. Lyon, B. B. V.: Ann. Int. Med. **9**:470, 1935.

5. Colman, H. C., and Marshall, C. R.: Lancet **1**:1709, 1907.

6. Hackh, I. W. D.: Chemical Dictionary, Philadelphia, P. Blakiston's Son & Co., 1929.

7. Davis, P. A.: J. A. M. A. **103**:962, 1934. Haigler.[2] Colman and Marshall.[5]

Haigler [2] reported 5 cases of acute poisoning in men aboard the U. S. S. *Lexington* who had inhaled fumes from a broken can of carbon tetrachloride in a poorly ventilated compartment. When found, all the men exposed presented evidence of deep anesthesia. All recovered after several hours in fresh air.

In most reported cases of poisoning through inhalation of the fumes the effects are more chronic, the result of repeated exposures over a long period of time. Mauro [8] reported 2 cases of chronic poisoning from the use of carbon tetrachloride as a cleaning fluid, in men working over a large vat. Both the patients showed jaundice, and their livers were large and tender; both had albumin and casts in the urine, moderate nitrogenous retention, edema and hypertension. McGuire [9] reported 7 cases in a dry cleaning establishment using a 33.3 per cent mixture of carbon tetrachloride in a large vat. In most of his cases the poisoning was mild, but enlargement of the liver and jaundice were noted in 2, and in 1 case there was severe "acute glomerular nephritis" as well; all the patients recovered. Wirtschafter [10] reported 5 cases of toxic amblyopia from the inhalation of carbon tetrachloride fumes in a dry cleaning establishment. The poisoning was manifest by concentric constriction of the visual fields without central scotoma. Recovery followed promptly on cessation of the exposure.

Smyth and Smyth [11] determined the concentrations of the vapor in plants manufacturing, using or repacking carbon tetrachloride and compared the values with the results of physical examinations of men working in the plants studied. The men chosen for the studies had been working in an environment of carbon tetrachloride for at least ten years. Among 8 men working in concentrations above 100 parts per million, 2 had restriction of their visual fields. Another's blood calcium was below 7 mg. per hundred cubic centimeters of blood. One had a twenty-four hour output of urinary nitrogen above 35 Gm. Among 73 workers in an atmosphere averaging 100 parts of carbon tetrachloride per million, 18 had icterus indexes above 8; 12 had low blood calcium; 5 had indirect van den Bergh reactions "above 0.2 per hundred cubic centimeters"; 7 had restricted visual fields, and 13 showed variations in the blood count (especially lymphocytosis or monocytosis). None of the 96 workers examined was seriously ill. Smyth and Smyth [11] stated that carbon tetrachloride in the concentration of 100 parts per million is barely perceptible by smell to one on first entering a room, and that any concentration above this level calls for more ventilation.

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8. Mauro, G.: Clin. med. ital. **61**:192, 1930.
9. McGuire, L. W.: J. A. M. A. **99**:988, 1932.
10. Wirtschafter, Z. T.: Am. J. Pub. Health **23**:1035, 1933.
11. Smyth, H. F., and Smyth, H. F., Jr.: J. A. M. A. **107**:1683, 1936.

The health hazard resulting from the industrial use of carbon tetrachloride was recognized in France in 1932,[12] and the Société des médecins des hôpitaux de Paris demanded that carbon tetrachloride be listed among the dangerous poisons as a result of 2 cases, with a death, in the dye industry. There is at the present date no federal statute regulating the use of carbon tetrachloride in the United States.[13]

Besides its use in commercial dry cleaning fluids, carbon tetrachloride is also a common ingredient of home cleaning agents (Carbona, Energine, Quick as a Wink, and others). Its use in the home has resulted in a number of fatalities from inhalation. Gonzales [14] reported a fatality from "Quick as a Wink" used in this way. Autopsy disclosed no anatomic cause for the death, but chemical examination showed a high concentration of carbon tetrachloride, particularly in the lungs. Two editorials in *The Journal of the American Medical Association* [15] pointed out the danger in the use of home dry cleaning agents in a poorly ventilated place. These difficulties and hazards are minimized, of course, in the commercial dry cleaning plants using carbon tetrachloride as a cleaning agent by the installation of proper apparatus for ventilation and by otherwise minimizing exposure.

Colman and Marshall [5] reported in 1907 the collapse of a woman following the use of carbon tetrachloride as a "dry shampoo". The woman recovered. At least one death has resulted from its use in this manner.[16]

In addition to intoxication through inhalation of the vapors of carbon tetrachloride, poisoning has resulted from accidental ingestion of this chemical. MacMahon and Weiss [17] reported the case of a 34 year old man who frequently became intoxicated on hair tonic and denatured alcohol. After a three day debauch he drank 1 ounce (31 cc.) of Carbona. Five days later he died, and autopsy showed central necrosis of the liver and macroscopic fat in the pulmonary artery. Lehnherr [18] reported the case of a 38 year old patient suffering from chronic alcoholism who drank from 4 to 5 ounces (125 to 155 cc.) of

12. Poisoning from Carbon Tetrachloride, Foreign Letters (Paris), J. A. M. A. **99**:1276, 1932.

13. Calvery, H. O., Chief of the Division of Pharmacology, Food and Drug Administration, U. S. Department of Agriculture: Personal communication to the author, Feb. 5, 1938.

14. Gonzales, T. A.: J. A. M. A. **106**:286, 1936.

15. Volatile Poisons in the American Home, editorial, J. A. M. A. **101**:1238, 1933; The Hazards of Dry Cleaning, editorial, ibid. **101**:1970, 1933.

16. Luce, R. V., and Hamilton, A.: Industrial Poisons Used in the Rubber Industry, Bulletin 179, United States Department of Labor, Bureau of Labor Statistics, 1909; cited by Hamilton.[3]

17. MacMahon, H. E., and Weiss, S.: Am. J. Path. **5**:623, 1929.

18. Lehnherr, E. R.: Arch. Int. Med. **56**:98, 1935.

carbon tetrachloride, mistaking it for alcohol. Jaundice appeared on the third day, and the icterus index reached 55 on the fifth day, thereafter falling slowly to normal with recovery. Heyl [19] reported a case of intoxication in a 2 year old boy who accidentally swallowed from 1 to 25 cc. of Energine (90 per cent carbon tetrachloride). The outstanding symptoms were slight jaundice, ascites, peripheral edema and anuria for several days. Three months later the child was still not well, but the galactose tolerance test and the phenolsulfonphthalein test were within normal limits.

Besides the occasional accidental poisoning resulting from the use of carbon tetrachloride in industry and the home, several fatalities have resulted from its use in the treatment of hookworm infestation. In 1921 Hall [20] introduced carbon tetrachloride for the removal of hookworms, recommending a dose of 3 cc. In 1922 Lake [21] gave monkeys from ten to forty times the anthelmintic dose recommended by Hall, on a basis of compared body weights, and noted no clinical symptoms or gross or microscopic changes in the liver or kidney which he could ascribe to the drug. Fatalities and dangerous intoxications following the use of the drug in the field occurred, however, although rarely.[22] Constantly autopsy in fatal cases disclosed evidence of central necrosis of the hepatic lobules or acute yellow atrophy of the liver. Almost every one of the fatalities occurred after an alcoholic debauch, and it was subsequently recommended that the drug not be used under such circumstances.

In an attempt to study the toxicity of the drug in man under controlled conditions, the drug was given to a number of condemned prisoners in approximately the dose recommended for therapeutic purposes.[23] Autopsy disclosed in most of these no remarkable effects on the liver or on the kidney, but fatty degeneration of the liver cells was noted in some of them.

EXPERIMENTAL STUDIES WITH CARBON TETRACHLORIDE

The toxicity of carbon tetrachloride has been extensively studied on experimental animals by many workers. It has been given by

19. Heyl, H. L.: J. Pediat. **9**:662, 1936.

20. Hall, M. C.: J. A. M. A. **77**:1641, 1921.

21. Lake, G. C.: Pub. Health Rep. **37**:1123, 1922.

22. (*a*) Smillie, W. G., and Pessôa, S. P.: Am. J. Hyg. **3**:35, 1923. (*b*) Lambert, S. M.: J. A. M. A. **80**:526, 1923. (*c*) Lamson, P. D.; Minot, A. S., and Robbins, B. H.: ibid. **90**:345, 1928. (*d*) Phelps, B. M., and Hu, C. H.: ibid. **82**:1254, 1924.

23. Docherty, J. F., and Burgess, E.: Brit. M. J. **2**:907, 1922. Nicholls, L., and Hampton, G. G.: ibid. **2**:8, 1922. Docherty, J. F., and Nicholls, L.: ibid. **2**:753, 1923. Leach, C. N.: J. A. M. A. **78**:1789, 1922.

mouth, by inhalation, by injection into the portal vein, by injection into the inferior vena cava, subcutaneously, intraperitoneally, by rectum and by rubbing into the skin. The variations in the composition of the blood under various circumstances of poisoning have been investigated. Regeneration of the liver after experimental damage by carbon tetrachloride has been the problem of other workers. Summarized in brief, the following results have been obtained:

Carbon tetrachloride is absorbed readily from the small intestine and is eliminated in expired air.[24] If fat is taken along with the carbon tetrachloride, the drug—a powerful fat solvent—is carried to the thoracic duct and directly to the lungs. The symptoms produced then are very similar to those following inhalation of the drug,[25] and recovery follows more rapidly. Experimentally, the symptoms following administration of carbon tetrachloride by mouth with fat or by inhalation are those of excitability followed by coma. If death occurs, it comes on much earlier than under ordinary circumstances of taking the drug by mouth; at autopsy lesions of the liver are less conspicuous than are the hemorrhages scattered throughout the body and focal bronchopneumonia.[26] If taken by mouth without fat, the drug is absorbed into the portal vein and passed to the liver, and elimination (via expired air) is much slower.[24a] The symptoms of intoxication when the drug is administered by mouth without fat are more insidious. Necrosis of liver cells is constant.[27] In dogs this first becomes apparent about twelve to twenty-four hours after the administration of the drug and becomes more severe until about forty-eight hours. Mild cloudy swelling or fatty degeneration of the epithelial cells of the convoluted tubules of the kidney has been noted by some workers.[28] Necrosis of the adrenal glands is said to occur in guinea pigs [22d] but is absent in dogs.[27a]

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24. (*a*) Robbins, B. H.: J. Pharmacol. & Exper. Therap. **37**:203, 1929. (*b*) Wells, H. S.: ibid. **25**:235, 1925.

25. Lamson, P. D.; Gardner, G. H.; Gustafson, R. K.; Maire, E. D.; McLean, A. J., and Wells, H. S.: J. Pharmacol. & Exper. Therap. **22**:215, 1923. Robbins.[24a]

26. Biancalini, A.: Arch. ital. di sc. farmacol. **3**:116, 1934.

27. (*a*) Gardner, G. H.; Grove, R. C.; Gustafson, R. K.; Maire, E. D.; Thompson, M. J.; Wells, H. S., and Lamson, P. D.: Bull. Johns Hopkins Hosp. **36**:107, 1925. (*b*) Schultz, E. W., and Marx, A.: Am. J. Trop. Med. **4**:469, 1924. (*c*) Cameron, G. R., and Karunaratne, W. A. E.: J. Path. & Bact. **42**:1, 1936. (*d*) Lacquet, A. M.: Arch. Path. **14**:164, 1932. (*e*) Meyer, J. R., and Pessôa, S. B.: Am. J. Trop. Med. **3**:177, 1923.

28. Gardner and others.[27a] Meyer and Pessôa.[27e]

Similar damage of the liver follows inhalation of carbon tetrachloride vapor in low concentrations over a long period of time or repeated inhalation of larger doses.[29]

Severe damage of the liver by carbon tetrachloride is followed by hypoglycemia in dogs.[30] Marked hypoglycemia also follows total removal of the liver in the dog.[31] Animals on a high carbohydrate diet prior to administration of carbon tetrachloride show less susceptibility to injury of the liver by the drug.[32] That calcium metabolism is of prime importance in carbon tetrachloride intoxication has been noted by many workers. Lamson and his co-workers [25] noted that dogs on a mixed diet can be given large single doses (as much as 250 cc.) by mouth without evidence of intoxication, although lesions of the liver occur. It was subsequently suggested [33] that the lack of symptoms of intoxication was due to the supplying of adequate calcium in the diet.

A possible relationship between the toxicity of carbon tetrachloride and the ingestion of alcohol has already been suggested. Wells [24b] showed that the presence of 50 per cent alcohol in an isolated intestinal loop of the dog does not hasten absorption but that with higher concentrations of alcohol absorption is materially accelerated. It has been shown experimentally that hepatic damage is more intense when carbon tetrachloride is given with alcohol.[34]

CARBON TETRACHLORIDE AND CIRRHOSIS OF THE LIVER

In addition to the association of carbon tetrachloride with acute yellow atrophy or toxic necrosis of the liver, which can be accepted as proved, there is much evidence that long-continued exposure to the drug may result in cirrhosis of the liver. Butsch [35] reported a case of cirrhosis of the liver, with ascites, following exposure to carbon tetrachloride vapors in dry cleaning; likewise, Poindexter and Greene.[36] In the case reported by Butsch the exposure was of six months' duration; in that reported by Poindexter and Greene it was of five years' duration. Lyon [4] reported a case of probable cirrhosis of the liver following almost daily use for

29. Smyth and Smyth.[11] Biancalini.[26] Gardner and others.[27a]

30. (*a*) Minot, A. S., and Cutler, J. T.: J. Clin. Investigation **6**:369, 1928. (*b*) Minot, A. S.: J. Pharmacol. & Exper. Therap. **43**:295, 1931. (*c*) Cutler, J. T.: ibid. **45**:209, 1932.

31. Mann, F. C., and Magath, T. B.: Arch. Int. Med. **30**:73, 1922.

32. Davis, N. C.: J. M. Research **44**:601, 1924.

33. Minot, A. S.: Proc. Soc. Exper. Biol. & Med. **24**:617, 1927. Lamson and others.[22c] Minot and Cutler.[30a] Cutler.[30c]

34. Lamson and others.[22c] Lamson and others.[25] Gardner and others.[27a] Minot and Cutler.[30a]

35. Butsch, W. L.: J. A. M. A. **99**:728, 1932.

36. Poindexter, C. A., and Greene, C. H.: J. A. M. A. **102**:2015, 1934.

five years of a hair tonic containing precipitated sulfur suspended in carbon tetrachloride. It has been shown experimentally that carbon tetrachloride can produce cirrhosis. Moon [37] stated that carbon tetrachloride produces typical Laënnec's cirrhosis, especially when combined with alcohol and when the diet is rich in fats and poor in carbohydrates. After the cirrhosis is once established in the experimental animal, it tends to progress slowly without further administration of the poison. Marked ascites, esophageal varices and hemorrhage can be produced. Goodpasture, studying the livers of dogs poisoned with carbon tetrachloride by Lamson and Wing,[38] believed the cirrhotic lesion to be different from that found in human atrophic cirrhosis but added that " . . . a more advanced lesion of this type would be very difficult to distinguish from the advanced human lesion." Cameron and Karunaratne [27c] quoted Fessinger as saying that there is one form of cirrhosis only, no matter what the causative agent. Cameron and Karunaratne agreed with this statement but considered that the intensity of the effect varies with the nature and the amount of the poison administered. Radhakrishna Rao [39] stated that the cirrhosis produced experimentally by carbon tetrachloride has the appearance of toxic cirrhosis.

REPORT OF THREE CASES OF ACCIDENTAL POISONING BY CARBON TETRACHLORIDE

In view of the extensive experimental studies made with carbon tetrachloride and of the scattered cases of human poisoning, it seems worth while to report 3 cases of accidental poisoning by the drug, in which autopsies were made.

On Aug. 30, 1935, a partially empty can of "roach poison" containing a mixture of carbon tetrachloride (25 per cent) and ethylene dichloride (75 per cent) [40] was discarded by a local bakery and placed on the city dump then used for the disposal of garbage in Charleston, S. C. On this day a small group of Negroes, looking for whatever they might find of value, found the partially empty tin. They mistook the pungent aromatic odor of the contents for that of whisky or alcohol, and 3 of them proceeded to drink it. The exact amount taken by each is not known, but the 38 year old Negro believed that he drank about half a pint (235 cc.). The entire amount (about 2 gallons [7.5 liters]) was not consumed, the balance being hoarded away, presumably for later consumption. On the instigation of the hospital authorities, the remainder was seized by the police a few days later, apparently before any one else had tasted it. One by one the

37. Moon, V. H.: Arch. Path. **18**:381, 1934.

38. Lamson, P. D., and Wing, R.: J. Pharmacol. & Exper. Therap. **29**:191, 1926.

39. Radhakrishna Rao, M. V.: Indian J. M. Research **23**:1007, 1936.

40. Subsequent analysis of a specimen of the fumigant was made by Mr. J. E. Aull, of the Department of Biochemistry, who found that the analysis claimed was substantially correct. No phosgene was found, and only a small amount of chloroform was present.

patients were brought to the Roper Hospital. The first patient died within from six to eleven hours after taking the poison, never rousing from a deep coma. The second was admitted in a stupor and died within from sixty-five to seventy hours after ingesting the mixture. The third died one hundred and fifty hours after ingestion of the poison. A more complete record of each case follows.

Case 1.—A 65 year old Negress was found by the police in a coma after having taken an unknown amount of the "roach poison" a few hours before. An odor was detected on the breath, described as similar to chloroform. (This was before the nature of the poison was known.) The rectal temperature was 97 F., the pulse rate 108 and the respiratory rate 44 per minute. The skin was cold and clammy, covered with perspiration. The pupils were dilated and reacted only slightly to light. The respirations were shallow, and there were scattered ronchi over both pulmonary fields. There was no control of the rectal and bladder sphincters. The blood urea nitrogen was 44 mg. per hundred cubic centimeters; the creatinine 2.6 mg.; the sugar 237 mg. She died about six to eleven hours after taking the poison and four hours after admission to the hospital.

Autopsy (two days after death) showed scattered hemorrhages in the serosal coverings of the abdominal viscera, in the lungs and about the pancreas, as well as atherosclerosis of the aorta and small granular kidneys. There was a definite odor of carbon tetrachloride to the gastric contents but not to the cut surfaces of the lungs. No other abnormalities were noted grossly. Microscopic sections revealed no evidence of necrosis of liver cells (figs. 1 *A* and 2 *A*) and there was no stainable fat in the liver cells (osmic acid and scarlet red). The lungs showed numerous hemorrhages into the alveoli. The kidneys presented marked arteriosclerosis affecting especially the smaller arteries and resulting in hyalinization of many glomeruli. The cells of the convoluted tubules appeared normal, and no fat was demonstrable in them. Sections of the heart, spleen, pancreas, adrenals and stomach appeared normal. There was no demonstrable fat in the lungs. In brief, the only changes that could be assigned to the intoxication were the scattered hemorrhages in the tissues.

Case 2.—A 54 year old Negro was brought to the hospital in a stuporous state about sixty-three to sixty-eight hours after he had taken an unknown amount of the poison. He was vomiting frequently, the vomitus containing small quantities of blood. The temperature was 98 F.; the pulse rate, 90; the respiratory rate, 38 per minute; the blood pressure, 70 systolic and 40 diastolic. The pupils were small and reacted only slightly to light. The respirations were labored. There was no odor to the breath. No jaundice was noted. The results from the remainder of the physical examination were negative. The blood showed a hemoglobin content of 85 per cent and 9,800 white cells per cubic millimeter, with 71 per cent polymorphonuclears, 28 per cent lymphocytes and 2 per cent monocytes. A specimen of urine could not be obtained because of a stricture of the posterior end of the urethra. The blood urea nitrogen was 63 mg. per hundred cubic centimeters; creatinine, 8.4 mg., and sugar, 40 mg. Muscular twitchings developed and death occurred two and a half hours after the patient's admission to the hospital or about sixty-five to seventy hours after the poison had been taken.

Autopsy was performed immediately after death. There was moderate jaundice of the scleras and of the subcutaneous tissues but no definite edema. There was no fluid in the abdominal cavity. Many small hemorrhages were noted in the retroperitoneal tissues, in the mesocolon, in the stomach, in the walls of the gallbladder, about the kidneys, in the epicardium and endocardium and in other tissues. No odor was noted to the gastrointestinal contents or to the other viscera. The liver

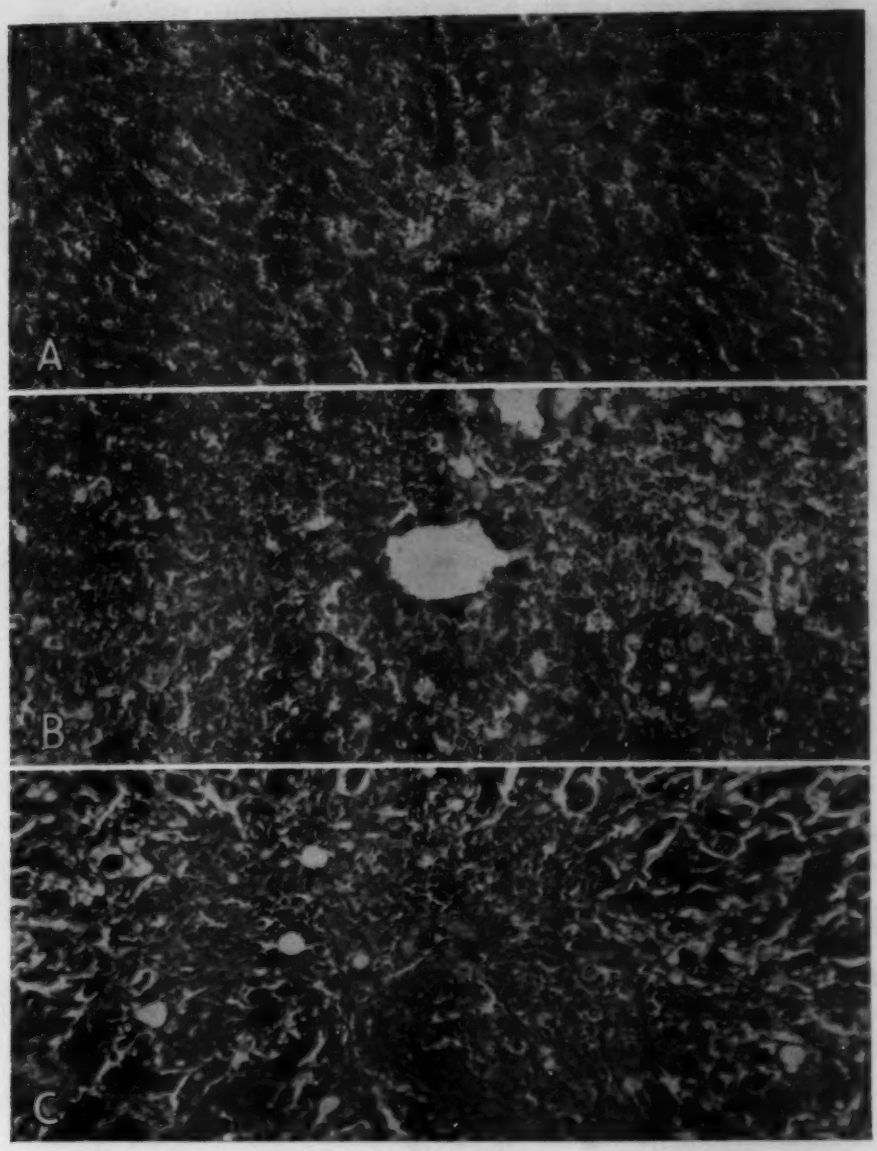

Fig. 1.—*A*, normal liver in case 1. The patient died about eight hours after ingestion of carbon tetrachloride; hematoxylin and eosin; about × 100. *B*, acute necrosis of the liver (case 2) about sixty-eight hours after poisoning by carbon tetrachloride; hematoxylin and eosin; about × 100. *C*, healing central necrosis of the liver (case 3) about one hundred and fifty hours after poisoning; hematoxylin and eosin; about × 100. Note the marked thickening of the wall of the central venule and the strands of regenerating cells about the area of central necrosis.

weighed 1,400 Gm. and showed a mottled reddish and yellow appearance through the capsule and on the cut surface. The liver tissue was friable, bled readily and was interpreted as necrotic throughout. The mucosa of the stomach was reddened by small hemorrhages, and there was a gastric ulcer 1.5 cm. in diameter near the pylorus. The mucosa of the remainder of the gastrointestinal tract was normal except for some edema and scattered hemorrhages. The other organs showed many small hemorrhages but no other gross lesions.

Microscopic examination showed hemorrhages in almost all the organs, with especial changes in the liver and kidneys. In the liver most of the parenchymal cells were destroyed and disintegrated, their places taken by blood cells (fig. 1 *B*). The necrosis was not confined to the central portions of the lobules, although these areas bore the brunt of the destruction. Liver cells were recognizable here and there in necrotic areas as large rounded globules of cytoplasm, studded frequently with tiny droplets of fat, as shown by osmic acid and scarlet red. Their nuclei were usually not visible or were represented by granules or small dark masses of basophilic material. Some viable liver cells were noted, especially about larger portal vessels; these cells showed swelling and vacuolation of their cytoplasm, with intact nuclei staining normally. These cells were scarce, however, and whole sections could be studied without noting a single group of such cells. Polymorphonuclear cells were present in considerable numbers, especially toward the peripheral regions of the lobules. Red blood cells were more numerous in the midzones of the lobules. Sections stained for reticulum fibrils according to the technic recommended by Gomori [41] showed a great reduction in the number of stainable fibrils and apparently marked disorganization, especially about the central zone of the lobule (fig. 2 *B*). There was no evidence of the formation of young "bile ducts." No mitoses or other evidences of regeneration of liver cells were to be found.

In the kidneys the greatest damage was effected in the convoluted tubules; the lining cells were greatly swollen, their cytoplasm was granular and the cell membranes were poorly defined (fig. 3 *B*). Many of these cells were completely necrotic and densely eosinophilic. Occasional fat droplets were noted in them but were not conspicuous. The epithelial cells of the capsule of Bowman and those covering the glomerular tuft were markedly swollen, standing up as cuboidal cells with prominent nuclei. There was no evidence of necrosis in these cells, however. The capsular space in some instances contained fat droplets, staining well with osmic acid, and in others it showed a granular eosinophilic material, apparently albuminous fluid. The glomerular tufts proper did not appear to be swollen, and their vascularity and cell content appeared about normal; there was no tendency to the formation of eosinophilic fibrils as is frequently noted in glomerulonephritis. There was much granular eosinophilic material in the tubules of the kidney, and some contained red blood cells.

In the adrenals there was extreme vacuolation of some of the cells of the outer portion of the fascicular zone but no definite necrosis. In the pancreas there were small zones of necrosis here and there, which appeared to be related to obstruction of small ducts by interlobular hemorrhage. The mucosa of the stomach showed superficial cellular necrosis and hemorrhage, with little cellular exudation. Sections of the heart, spleen, lungs and small intestine appeared normal except for hemorrhages.

Case 3.—A 38 year old Negro took about half a pint of the poison. Fifteen minutes later he vomited a large amount of it and continued to vomit intermittently

41. Gomori, G.: Am. J. Path. **13**:993, 1937.

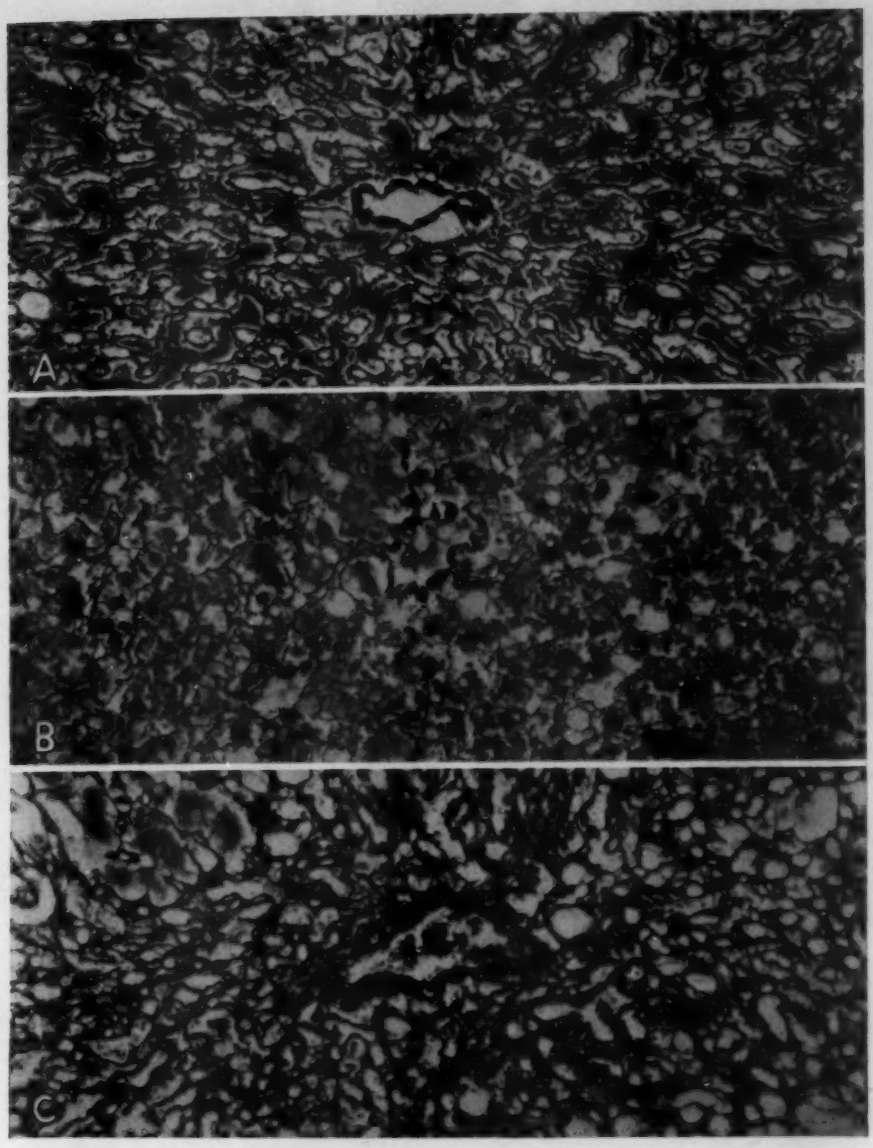

Fig. 2.—*A*, normal distribution of reticulum fibers in the liver in case 1; silver impregnation; about × 110. *B*, disruption of the reticulum in acute necrosis of the liver (case 2); silver impregnation; about × 110. *C*, condensation of reticulum about the central venule of a hepatic lobule, with thickening of the wall of this venule, in healing central necrosis of the liver (case 3); silver impregnation; about × 110.

until admitted to the hospital. He vomited no blood and did not think he had passed any blood by rectum. Shortly after taking the poison he noted burning pain throughout the abdomen. He became very weak but was not confined to bed all the time. There was some diminution in the amount of urine passed but no anuria and no hematuria. He was admitted to the hospital ninety-five hours after having taken the chemical. The temperature was 98.4 F. (rectal); the pulse rate, 100; the respiratory rate, 24 per minute; the blood pressure, 155 systolic and 75 diastolic. The pupils were small and reacted normally. The conjunctivae and scleras were icteric. The lungs were clear, and the heart action was normal. The edge of the liver was palpated about 4 cm. below the costal border, in the right midclavicular line. The tendon reflexes were slightly hyperactive. Urinalysis showed a large amount of albumin but no sugar, acetone or casts; a few red blood cells and about 100 leukocytes per high power field were noted. The hemoglobin was 87 per cent (Dare), and the leukocytes of the blood numbered 6,400 per cubic millimeter, with 70 per cent polymorphonuclears, 27 per cent lymphocytes, 2 per cent monocytes and 1 per cent eosinophils. The coagulation time was eleven minutes; the calcium time, eight minutes. The urea nitrogen of the blood was 54 mg. per hundred cubic centimeters; creatinine, 8.2 mg.; sugar 82 mg.; serum calcium, 8 mg. The direct van den Bergh test gave a strongly positive reaction, estimated as 4 plus, and the icterus index was 62. Dextrose in solution and calcium gluconate were given by vein. On the day after admission the coagulation time was five minutes; the calcium time, four minutes; the bleeding time, two and one-half minutes. The blood platelets numbered 225,000 per cubic millimeter. The blood sugar was 149 mg. per hundred cubic centimeters of blood; cholesterol, 130 mg.; serum calcium, 7.6 mg. On the day of death the blood sugar was 110 mg. per hundred cubic centimeters of blood, and the carbon dioxide–combining power was 72 volumes per cent. The patient was able to retain little taken by mouth, and the intravenous administration of fluid and dextrose was continued. On the day of death the respirations became more labored; the temperature rose to 101.6 F. (rectal), and the patient died fifty-five hours after admission to the hospital and one hundred and fifty hours (six and a quarter days) after ingesting the poison.

Autopsy was performed twelve hours after death. There was moderate edema of the legs and face, with pronounced chemosis of the conjunctivae and numerous subconjunctival hemorrhages. There was marked jaundice of the subcutaneous tissues. About 25 cc. of clear straw-colored fluid was present in the abdominal cavity. Hemorrhages were numerous in the looser tissues of the mesentery, lungs, pancreas and about the kidney. Pneumonia was present in the lower lobes of the lungs. Syphilitic aortitis was present, involving one of the commissures of the aortic valve. The liver weighed 1,080 Gm., and its capsule was wrinkled and opaque. The parenchyma was very soft and friable, deeply jaundiced and mottled, and the normal architecture of the organ could not be made out. The mucosa of the stomach was slightly reddened but intact, and the remainder of the gastrointestinal tract appeared normal. The kidneys were moderately enlarged, weighing about 225 Gm. each. The edges of the kidney bulged when sectioned, and the whole parenchyma appeared swollen, especially the cortex. The urine of the bladder was the color of amber and clear. The other organs appeared grossly normal.

Microscopically, the essential lesions were in the liver and kidneys. In the liver there was evidence of extensive destruction; the old liver cells from the central portions of the lobules had been almost completely removed, leaving only the collapsed stroma, with a few lymphocytes and irregular collections of erythrocytes in and about the central venules (fig. 1 *C*). Individual lobules were

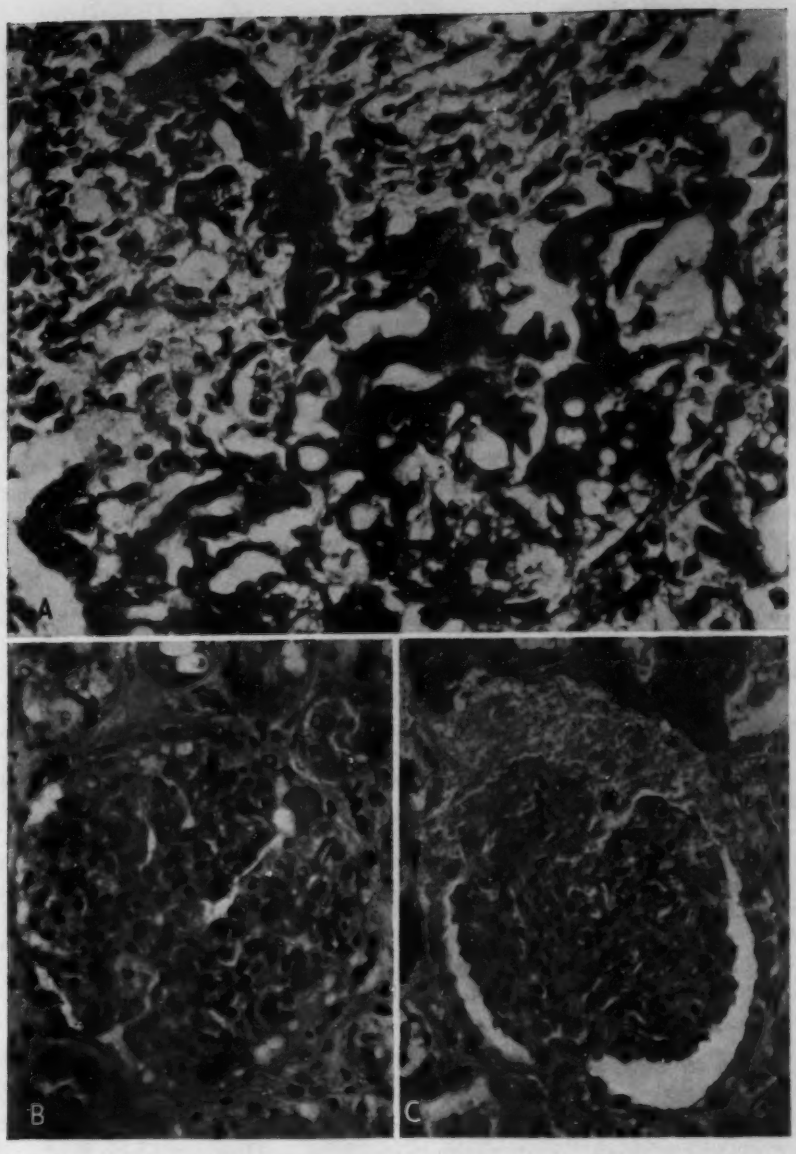

Fig. 3.—*A*, regeneration from liver cells in healing central necrosis of the liver (case 3), with a tendency for the new cells to form tubules; hematoxylin and eosin; about × 300. *B*, beginning necrosis of cells of renal tubules, albuminous fluid in Bowman's space and swelling of epithelial cells covering a glomerular tuft sixty-eight hours after poisoning (case 2); hematoxylin and eosin; about × 180. *C*, necrosis of cells of convoluted tubules and albuminous fluid in Bowman's space one hundred and fifty hours after poisoning (case 3); hematoxylin and eosin; about × 180. The black granules in the glomerulus are the pigment relic of an old malarial infection.

considerably reduced in size. The central venules showed definite thickening of their walls, so that the lumens appeared slightly narrowed. The liver cells about the periphery of each involved lobule were large, and the nuclei were large and generally hyperchromatic; a few mitotic figures were to be seen. Their cytoplasm was very granular and somewhat more eosinophilic than normal. Where the liver cells bordered on the necrotic central zone, the cells were even larger, and many contained a great number of bubble-like vacuoles, which stained with osmic acid and scarlet red. Fat vacuoles were less numerous peripherally in the hepatic lobules, where the cells appeared normal. Projecting from these liver cells and appearing to have arisen from them were budlike projections of elongated cells, apparently epithelial, having a tendency to form tubules (fig. 3 *A*). These were projected toward the centers of the hepatic lobules and were not to be found in the perilobular spaces, where the bile ducts proper appeared normal and showed no budding. Continuity between the larger liver cells, with deeply eosinophilic cytoplasm, and the smaller cells of these tubules, with scant cytoplasm, was definite. The impression was gained that they represented an effort on the part of the viable liver cells to replace their kind, lost from the center of the lobule. None of these tubular spaces contained bile, but small granules of bile pigment were occasionally to be seen in the liver cells proper. Sections stained with silver showed a pronounced condensation of the reticulum about the central venule and an increase in the reticular fiber content of the wall of the central venule, while further toward the periphery of the lobule the reticulum was much more normally disposed (fig. 2 *C*).

Sections of kidney showed as a striking feature the collection of eosinophilic granular, apparently albuminous fluid material in the space of Bowman, with a flattening of the glomerular tuft to one side of the space (fig. 3 *C*). The epithelial cells covering the glomerulus and lining Bowman's capsule were slightly more prominent than usual and here and there appeared to be reduplicated, but there was no suggestion of crescent formation. The glomerular tufts proper appeared fairly normal; they did not contain an excess of leukocytes, and the blood flow in their capillaries appeared fairly normal. The convoluted tubules showed swelling of their tall epithelial cells, with loss of cell outline and increased granularity of cytoplasm, and there was definite cellular necrosis. Most of the tubules contained albuminous material identical with that noted in Bowman's space, but there were no formed casts and there was only an occasional blood cell in them.

The adrenal gland was generally normal, but in some portions of the zona fasciculatis there was shriveling of the cell nuclei and almost complete vacuolation of the cytoplasm, giving an appearance as of small linear areas of necrosis.

COMMENT

The lesions encountered in the cases reported here are those to be expected with carbon tetrachloride alone. However, since all the aliphatic halogens are known to have a somewhat similar effect, it is possible that ethylene dichloride was also a factor.

Hueper and Smith [42] reported a case of accidental poisoning by ethylene dichloride, death occurring twenty-two hours later. Autopsy disclosed "moderate fatty degeneration" of liver cells peripherally in the liver lobules but no necrosis. The most striking lesions were multiple

42. Hueper, W. C., and Smith, C.: Am. J. M. Sc. **189**:778, 1935.

hemorrhages, ulcerative colitis, and necrosis and calcification of the epithelium of the renal tubules, comparable to that seen in poisoning from mercury bichloride.

Sayers and his associates [43] exposed guinea pigs to the vapor of ethylene dichloride for varying periods of time. In animals examined post mortem from one to eight days later, there was no evidence of hepatic necrosis, and the conspicuous observation was "degenerative changes in the kidneys." Apparently necrosis of the liver is not an important feature of poisoning by ethylene dichloride.

Hamilton [44] stated that it is safe to assume that carbon tetrachloride was the chief toxic agent in the cases reported here but that "when there is a mixture of two closely related bodies, one must assume that each has a share, though we do not know how great a share."

The contrast between cases 1, 2 and 3 is striking. The first patient, who died between six and eleven hours after taking the poison and who remained in a coma for the duration of her stay in the hospital, showed no lesions at autopsy except scattered hemorrhages throughout the body. This death is interpreted as being more or less an anesthetic death, such as may occur from the inhalation of carbon tetrachloride or from ingestion of the drug after a fatty meal (Lamson and his collaborators [25] did not produce narcosis in dogs even when they gave massive doses of carbon tetrachloride by mouth unless they gave it with cream). It is conceivable that a single large dose of the drug, i. e., larger than is compatible with life for more than a few hours, may produce death in this way even if taken by mouth. The dosage of the drug and the nature of the food taken are not known in this case. That no lesions of the liver occurred in the patient is compatible with the observations of Gardner and his co-workers [27a] that central necrosis does not occur until after twelve hours.

(Mitochondrial staining was contemplated for this case but was not done because of the long period between death and autopsy and because of lack of experience with mitochondrial studies. Lacquet [27d] reported mitochondrial changes in the liver of the rat within three hours after 0.25 cc. of carbon tetrachloride was given by mouth.)

Of some interest is the high level of blood sugar (237 mg. per hundred cubic centimeters of blood) encountered in case 1. Hyperglycemia and glycosuria are known to occur occasionally after prolonged general anesthesia. Might it be that even before necrosis of liver cells is produced by the poison there is an effect on the liver cells which sets free stored glycogen, resulting in temporary hyperglycemia, and that

43. Sayers, R. R.; Yant, W. P.; Waite, C. P., and Patty, F. A.: Pub. Health Rep. **45**:225, 1930.

44. Hamilton, A.: Personal communication to the author.

this is followed later by hypoglycemia as the stores of glycogen are depleted? The occurrence of hypoglycemia after carbon tetrachloride poisoning and after total removal of the liver has already been alluded to. The observation is borne out by the low levels of blood sugar in cases 2 and 3 (40 mg. and 82 mg. per hundred cubic centimeters of blood, respectively) after the establishment of definite damage of the liver. Using Best's carmine stain on pyroxylin sections, I was unable to demonstrate glycogen in the liver in cases 1 and 2, but there was some glycogen demonstrable in the liver in case 3; the patient had received large quantities of dextrose by vein, and the liver had already passed the point of most severe damage. (It is realized that the failure to stain glycogen in case 1 is of no actual significance, since the autopsy was not made until two days after death.)

In case 2 damage of the liver was well established in from sixty-five to seventy hours after the poison was taken, but there was yet little evidence of removal of dead liver cells, although phagocytosis had begun. There was no evidence of attempted regeneration of liver cells or of other structures.

In case 3 destruction of liver cells had passed its peak; the necrotic cells and tissue detritus had been almost completely removed, and regeneration was well under way. While this is probably somewhat earlier (six and a quarter days) than regeneration of liver is generally thought to occur, it coincides fairly closely with the time of onset of regeneration in experimental animals. Phelps and Hu [22d] noted evidence of regeneration of liver cells in the guinea pig after forty hours. Gardner and his co-workers [27a] noted "healing central necrosis" in dogs seven days after the animals received 4 cc. of carbon tetrachloride per kilogram. Cameron and Karunaratne [27c] noted that repair was active in the rat's liver three days after the rat was given a single toxic dose of carbon tetrachloride. It is probable that the speed with which regeneration occurs depends on the severity of the damage to the liver cells and on the ratio of destroyed to viable cells.

In case 3 it seems that regeneration of liver cells is taking place entirely from liver cells and that the bile ducts are playing no part in the process. Numerous strandlike projections of cells can be seen arising from the liver cells about the periphery of the lobule and projecting into the central portion of the lobule, and an occasional mitotic figure is to be seen in these cells. Frequently these cells show a definite tendency to form tubular structures, but even then the cells can be traced to liver cords, while the bile ducts at the periphery show no evidence of budding or other activity. This evidence of direct regeneration of liver cells from other liver cells cannot be taken as conclusive evidence that regeneration always occurs from liver cells, since in cases in which more complete destruction of liver cells has occurred, it is conceivable

that proliferation from the bile ducts may be possible. However, it seems that proliferation from bile ducts is a later feature of regeneration than is direct regeneration of liver cells. That the tubular projections from the liver cells might at a later stage appear as bile ducts seems unlikely, since the structures noted in case 3 are toward the centers of the lobules rather than in the peripheral regions, where bile ducts are usually seen in cases of longer standing and in cases of toxic cirrhosis.

(Studies of liver cell regeneration have yielded varying opinions from time to time. MacCallum [45] believed that regeneration of liver cells took place from liver cells proper when enough of them remained but that it might occur from bile ducts. Milne [46] stated that ". . . regeneration results from a proliferation of liver cells directly, and with no transition in type of cell"; he stated further that the bile duct–like structures in areas of liver damage ". . . seem to be formed from a becoming evident of the delicate, normal, bile-conducting channels which extend between the liver cells and interlobular bile ducts." Fishback [47] stated that "The consensus of opinion is that bile ducts proliferate and serve as a source of new hepatic cells, especially when the destruction of parenchyma has been severe." Cameron and Karunaratne [27c] believed that the bile ducts played only a small part in the regeneration of liver cells, and in their experiments on rats, in which they used a single toxic dose of carbon tetrachloride, they noted no bile duct proliferation at the fourteenth day, when regeneration of the liver appeared to be complete. Moon [27] stated that in repair following injury all hepatic structures [liver cells, bile ducts, fibrous tissue, Kupffer cells, vascular structures] responded but that regeneration of liver cells occurred from those liver cells which escaped destruction.)

That destruction of the reticulum occurs along with destruction of the parenchymal cells of the liver seems to have received little comment. Radhakrishna Rao [48] noted in so-called "infantile biliary cirrhosis" occurring among children of India that disorganization of the reticulum of the sinusoidal bed about central venules occurred. In his studies with carbon tetrachloride on experimental animals [39] he noted "disorganization, collapse, condensation and sclerosis of the reticulum of the sinusoidal capillary bed around the hepatic terminal consequent to the necrosis of the parenchyma in the same area."

In the cases reported here, this effect was striking. In case 2, in which acute yellow atrophy of the liver was present, there was marked disintegration of the reticulum in the central portions of the lobules, where the effect on liver cells was also greatest. After the débris from

45. MacCallum, W. G.: J. A. M. A. **43**:649, 1904.
46. Milne, L. S.: J. Path. & Bact. **13**:127, 1909.
47. Fishback, F. C.: Arch. Path. **7**:955, 1929.
48. Radhakrishna Rao, M. V.: Indian J. M. Research **23**:68, 1935.

destruction of liver cells had been removed (case 3), a collapse of the reticulum occurred about the central venule. This condensation resulted in thickening of the walls of the central venule and in marked distortion of the sinusoids. That this effect is important seems likely; it may be the major factor in bringing about portal obstruction.

The nature and significance of the renal changes in these cases are somewhat more obscure. Gardner and his associates,[27a] working with dogs, commented on the accumulation of eosin-staining granular material, apparently albumin, within Bowman's capsule and noted moderate cloudy swelling of the epithelium of the convoluted tubules, without fatty change or necrosis, but added that the ". . . kidney lesions are not striking." Meyer and Pessôa[27c] noted fatty changes in the epithelial cells of the convoluted tubules in dogs. That the renal changes are of some importance is suggested by the clinical records submitted here. In cases 2 and 3 there was marked nitrogenous retention, and in case 3, a large amount of albumin, a few red blood cells and many leukocytes in the urine. There was also shown in case 3 some diminution of the urinary output. None of the sections from the kidneys of the patients showed the usual features of glomerulonephritis. There was, however, a large amount of albuminous material in the space of Bowman, as well as marked swelling of the epithelial cells of the capsule of Bowman and of the convoluted tubules. Little fat could be demonstrated by osmic acid or scarlet red. The lesions here bring to mind those mentioned by Schutz, Helwig and Kuhn[49] in the kidneys of patients dying "liver deaths" after operations in the upper part of the abdomen. Experimentally, these authors produced "secondary" "degenerative" changes in the kidneys, with nitrogenous retention, oliguria, albumin, casts and red blood cells in the urine, by ligating the hepatic artery and then traumatizing the liver. They concluded that some toxin was probably elaborated from the liver which acted more or less specifically on the kidneys. In the cases reported here, the lesion seems to have been of "toxic" type, having its greatest effect on the epithelium of the proximal portion of the tubule; it seems likely that the responsible toxin was the carbon tetrachloride rather than some hypothetic substance thought to be elaborated by the damaged liver. That damage of the kidney occurs clinically in carbon tetrachloride poisoning has been referred to in an earlier portion of this paper.

SUMMARY

Three cases of accidental poisoning by carbon tetrachloride in man have been studied clinically and at autopsy. The deaths resulted from ingestion of a "roach poison" containing a mixture of carbon tetra-

49. Schutz, C. B.; Helwig, F. C., and Kuhn, H. P.: J. A. M. A. **99**:633, 1932.

chloride and ethylene dichloride, mistaken for an intoxicant. One victim died about eight hours after taking the drug and showed no definite lesions at autopsy. The second died about sixty-eight hours after ingesting the poison and at autopsy showed acute necrosis of the liver. The third patient died one hundred and fifty hours after poisoning and at autopsy presented active regeneration of lost liver cells.

A brief review of the toxicology and pharmacology of carbon tetrachloride is included, with emphasis on the results of studies in experimental animals. The probable relation of some cases of cirrhosis of the liver to poisoning by the drug is discussed.

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CALCIFICATION OF HYALINE CARTILAGE IN MAN

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Human cartilage can be classified histologically as fibrous, elastic and hyaline. The latter is found especially in the joints, in the framework of the larynx and bronchi and in the cartilages of the ribs.

Hansen's [1] fundamental investigations show that the structure of this hyaline cartilage, which arises from primitive mesenchymal tissue, is due to the differentiation of the protoplasm into endoplasm and exoplasm. By "endoplasm" or, more correctly, "the endoplasmic areas," is understood the nuclei of the hyaline cartilage.

The exoplasm is the transparent, hyaline type of protoplasm which occupies the area between the nuclei, and it is the substance in which the collagenous fibers are developped. The endoplasmic areas of nuclei excrete toward the exoplasm a substance which takes a deep basic stain. Thus in a section treated with the hematoxylin-eosin stain each of the endoplasmic areas appears to be surrounded by a blue ring, "the capsule of the cartilage cell."

In addition to describing normal hyaline cartilage, most of the handbooks of histology mention briefly the changes such cartilage undergoes with age.

In Friedmann's [2] description of the ordinary changes of senescence there is the statement that cartilage occasionally calcifies, but no further details of the process are included.

Orth [3a] described these changes somewhat more extensively in that he mentioned that in sections of older cartilage prepared with the hematoxylin-eosin stain there is a diffuse infiltration of calcium salts. No further details are given nor are there any illustrations. The same

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From the Pathologic Department of the Caroline Institute; Prof. Folke Henschen, Chief.

1. Hansen, F. C.: Researches on the Group of Connective Tissues: I. The Hyaline Cartilage, Copenhagen, Wilhelm Prior, 1900.

2. Friedmann, F.: Die Altersveränderungen und ihre Behandlung, Berlin, Urban & Schwarzenberg, 1902, chap. 14.

3. Orth, J.: (*a*) Cursus der normalen Histologie zur Einführung in den Gebrauch des Mikroskopes sowie in das practische Studium der Gewebelehre, Berlin, A. Hirschwald, 1878; (*b*) Spezielle pathologische Anatomie, ibid., 1887; (*c*) Compendium der pathologisch-anatomischen Diagnostik, nebst Anleitung zur Ausführung von Abductionen, ibid., 1876.

author [3b] described the beginning calcification of "the capsules of the cartilage cells" up to a stage where strands of calcium could be shown at the borderline between the two histologically differentiated parts of the hyaline cartilage.

A review of the special literature on calcification shows that Aschoff,[4] Beer,[5] Ribbert [6] and several others mentioned only briefly that "the rib cartilages are often calcified" and that the larynx in elderly men sometimes undergoes the same change.

The English, German and French handbooks in anatomy do not provide any further information on this subject.

Three papers have titles considerably more promising, namely, one, "Die Altersveränderungen der Invertebralscheiben," by Luschka,[7] one on the same subject by Uebermuth [8] and one entitled "Untersuchungen über die Struktur des normalen und des pathologisch veränderten Knorpels" by Tenderich.[9] In the monograph of Tenderich particularly, one would expect a detailed description of calcification in general, of calcification of rib cartilage in bronchial asthma and so on, but these subjects are scarcely mentioned and not discussed at all. Although Uebermuth's paper is interesting, the particular observations which he made cannot be applied to the entire cartilaginous system, for the invertebral disks consist partially of fibrous cartilage.

All the studies referred to have dealt with the entire system of hyaline cartilage, and no investigation seems to have been made of isolated parts of this system. It is true that Simmonds [10] published a paper in 1905 on the trachea in the elderly person, but his work was done without a microscope. Furthermore, Chiari,[11] Harbitz,[12] Kockel,[13] Pick,[14] Stade [15] and Virchow [16] in their reports of many cases of calcified pulmonary metastases mentioned calcification of the bronchial cartilages only in passing.

4. Aschoff, L.: (*a*) Pathologische Anatomie: Ein Lehrbuch für Studierende und Aertze, Jena, Gustav Fischer, 1936; (*b*) Ergebn. d. allg. Path. u. path. Anat. **8**:561, 1902.

5. Beer, E.: J. Path. & Bact. **9**:225, 1903-1904.

6. Ribbert, H., in Eulenburg, A.: Real-Encyklopädie der gesamten Heilkunde, Berlin, Urban & Schwarzenberg, 1907, pp. 14 and 464.

7. Luschka, H.: Virchows Arch. f. path. Anat. **9**:311, 1856.

8. Uebermuth, H.: Ber. d. sächs. Akad. **81**:111, 1929.

9. Tenderich, H. F.: Untersuchungen über die Struktur des normalen und des pathologisch veränderten Knorpels, Greifswald, J. Abel, 1892.

10. Simmonds, M.: Virchows Arch. f. path. Anat. **179**:15, 1905.

11. Chiari, H.: Wien. med. Wchnschr. **28**:6, 1878.

12. Harbitz, F.: Arch. Int. Med. **21**:139, 1918.

13. Kockel, R.: Deutsches Arch. f. klin. Med. **64**:332, 1899.

14. Pick, E. P.: Klin. Wchnschr. **14**:1, 1911; **33**:797 and 1017, 1917.

15. Stade, F. H.: Ueber Lungenverkalkung, Kiel, H. Fiencke, 1900.

16. Virchow, R.: Virchows Arch. f. path. Anat. **8**:1, 1855; **9**:618, 1856.

Thus, up to the present, papers on this subject have restricted themselves to the statement that hyaline cartilage in elderly persons sometimes undergoes asbestous or other forms of degeneration, loses its stainability and becomes calcified either by a mild incrustation of the "capsules of the cartilage cells" or by a slight diffuse infiltration of calcium salts.

SCOPE OF THE INVESTIGATION

The object of the present investigation was at first to clarify the way in which calcification of the cartilages of the trachea and bronchi takes place, especially in elderly women, in whom it is most often seen. From the very beginning of the study, however, it became clear that a number of other questions could not be avoided—for instance, that of the mechanics of the deposition of calcium in the rib cartilages and that of the friability of senile bone. Thus the subject broadened and turned out to be intimately associated with most of the other problems of calcium metabolism and its disturbance. The aim of the present study, was, however, limited as much as possible to the original plan.

MATERIAL

The cartilage for the investigation was obtained at autopsies in St. Erik's Hospital, Stockholm. Altogether, hyaline cartilage has been studied in 200 cases. The average age of the 97 men as well as of the 103 women from whom the material was obtained was around 62 years. The clinical diagnoses corresponded on the whole with the age groups. Thus the younger persons had, for the most part, succumbed to pulmonary tuberculosis or peritonitis secondary to some other disease, while the older persons had died of disease of the heart, blood vessels or lungs, lesions of the urogenital apparatus, malignant tumors or lesions of the central nervous system.

TECHNIC

At autopsy incisions were made in the cartilages of the ribs, bronchi, trachea and sometimes the larynx. In most cases it was simple to decide as to the presence of a large amount of solid calcium. Normal cartilage never contains calcium in this aggregated state. The pathologic calcium depots in the cartilage are felt with the knife in the same way as the calcium in tuberculous lymph nodes; that is to say, there is a distinct scraping sensation. In other instances there is increased resistance to the knife as if one were cutting hard wood. This is because calcium is precipitated in different ways in the tissues, as will be discussed farther on. The scraping sensation indicates the presence of larger amounts of calcium. However, it should not be forgotten that cartilage degenerates in the aged and that this helps to give rise to a still different sensation on incision. This degeneration reveals itself microscopically as a deficiency of the endoplasmic areas and a marked diminution in stainability, both of which are signs of poor tissue metabolism and vitality. Such cartilage is brittle and thin, exactly like the thin, dry skin of elderly persons, in which the juvenile elastin has changed into elacin.

This simple method of incision with a knife could be used in the diagnosis of calcification of cartilage only in cases in which calcification was absolutely certain. It is just these cases, however, which are easily recognized by the pathologist when he finds bronchi like stiff tubes and the cartilage in them scraping the knife.

The material which did not give these clear signs of calcification, and there was very little in this series which did not, was saved for further examination with the microscope and by other means. It turned out that many of these tissues showed the presence of pathologic [17] quantities of calcium. Samples of the cartilage obtained in the cases in which calcification was unmistakable were also examined further in this way. The tissues were prepared with the hematoxylin-eosin stain, the classic dye for calcium in the body.[18] In these preparations in cases in which calcification was sure, it was easy to see the different ways in which the tissues were infiltrated with calcium and the quantities of this foreign substance. The method gave a better conception of both the quality and the quantity of the deposited calcium than could have been obtained by tabellar grading of the results from microscopic investigation alone. Quantitative chemical analyses of all these tissues would have required altogether too much apparatus and certainly could not have provided more information than the microscopic sections did.

Finally microincineration offered a method of controlling the results already obtained. In this way the calcium could be demonstrated in the sections prepared with the hematoxylin-eosin stain and in the sharply reflected light from the residue in the ash. The procedure in the latter study was extremely simple. The specimen was incinerated on a piece of pyrex glass over a Bunsen burner.

All the preparations, both those showing calcification and those in which this change was in doubt or not found, were incinerated as described. It would be attractive to be able to show parallel series of hematoxylin-eosin sections and microincinerated sections representing all grades of calcification, from the mildest to the severest. In this way it would be possible to demonstrate, especially on the incinerated preparations, how the ash gradually concentrates around the endoplasmic areas, forming absolute lamellae in the exoplasm, and how outside the edges of the cartilage the ash encloses the cartilage in a ring of dead calcium. Unfortunately, only a few photographs can be submitted to give an idea of the actual conditions.

AREAS STUDIED

Cartilage.—Two cartilaginous areas have been studied, namely, that of the ribs and that of the trachea and bronchi. The latter area is taken to include all cartilage from the beginning of the larynx to the smallest cartilaginous rings or portions which are large enough for macroscopic examination.

Skeleton.—In each case a concept of the calcium conditions in the skeleton was obtained by examining the ribs and spinal column for possible osteoporosis or osteosclerosis. As porosis signifies calcium deficiency, and calcification of cartilage

17. The term "pathologic" as used in this connection requires explanation. Later on, the conclusion is drawn that the changes described must be regarded as characteristic of age. But, as senescence is a physiologic process, calcification must be physiologic in the same way as every other diminution in the vitality of the tissues. Presbyopia and muscular atrophy, for instance, are to a certain degree physiologic. However, each and every such condition signifies an injury to the organism as a whole and in this way becomes "pathologic." Here, as everywhere else, the boundaries between the normal and the abnormal are indistinct, and therefore the evaluation of this concept always becomes more or less subjective (Aschoff [4]).

18. Policard, A.: Bull. d'histol. appliq. à la physiol. **1**:26, 1924. Schultz-Brauns, O.: Verhandl. d. deutsch. path. Gesellsch. **26**:153, 1931.

the opposite, at least locally, a conscious attempt was made to demonstrate this contrast. As far as the skeleton is concerned, this was done in the usual way for detecting friability of bone. The ribs were exposed to the angle and the spinal column was divided with a broad chisel[19] from the fifth lumbar vertebra upward and then inspected and palpated. In order to acquire assurance in evaluation, both standard and definitely altered skeletal tissues were studied in this way. Normally the ribs are elastic, and a definite force is required to produce a fracture. In senile osteoporosis, however, a rib can be almost broken in pieces between the fingers, and the normal rich spongiosa and marrow are replaced by widespread grayish-yellow columns with little marrow in the interspaces. Moreover, the ribs are usually thin and flattened in this condition. Chiseling of the spinal column gives a good conception of the calcium content of the vertebral bodies, and the evaluation becomes still more accurate if palpation with a sound follows, for the instrument practically sinks by its own weight into the calcium-poor spongiosa.

The amount of calcium was graded from 1 to 3 plus, according to its degree. This grading was only empiric and entirely subjective. The difference between the cases was indeed usually marked.

Osteoporosis was also graded from 1 to 3 plus, according to its degree.

For calcified vessels only plus was used, for the only cases included were those in which the vessels were stiff with calcium and the inner surfaces covered with easily recognized calcified bodies. In all these cases the clinical diagnosis was severe arteriosclerosis. The vascular areas examined aside from the heart were the aorta, the celiac arteries, the splenic and renal arteries, the femoral artery and, if there was opportunity, the vessels at the base of the brain. Each case included here was one in which the usual signs of severe calcification were shown at least in the aorta and femoral artery.[20]

Finally, some other disturbances in calcium metabolism must be mentioned. The most important is that represented in gallstones. Every pathologist has seen what large masses of calcium can be deposited in the body in the form of such concrements. The same is true of renal stones and concrements in the lower portion of the urinary canal. Fat-calcium infarcts in the kidneys do not necessitate the presence of any great quantities of calcium, but they indicate some disturbance in the calcium metabolism. Similarly, calcified obliterative pericarditis and calcified rings around the cardiac ostia are well recognized.[21]

Calcified tuberculous lymph nodes have been omitted because they are to be found, in almost 100 per cent of cases, as a part of the primary complex. The primary and the contributory cause of death have also been left out, as they do not play any role in the understanding of the described lesions. Severe arteriosclerosis can be understood from the column headed "Calcified Vessels."

OBSERVATIONS

Figure 1 reveals that in 169 of the 200 cases studied there was a "pathologic" deposit of calcium in the cartilage; 84 of the persons with deposits of calcium in the cartilage were men, and 85, women. Their average age was about 64 years; that is to say, somewhat higher than

19. Henschen, F.: Zentralbl. f. allg. Path. u. path. Anat. **60**:55, 1934.

20. Gazert, R.: Deutsches Arch. f. klin. Med. **62**:390, 1899. Mönckeberg, J. G.: Virchows Arch. f. path. Anat. **121**:141, 1903.

21. Ipsen, J., Jr.: Nord. med. tidskr. **8**:1004, 1934.

the average age for the entire series. The lowest age at which calcification was demonstrated was 33 years. The patient was a man. The youngest woman was 45 years old. This age difference does not show that men age more rapidly than women in this respect, for the clinical diagnosis in the case of the man was pulmonary tuberculosis, the significance of which for calcification is discussed later. The first actual accumulation of cases in which deposits of calcium were found is for both series between 45 and 55 years of age. The curve then rises both as to the quantity of calcium and as to the number of areas involved.

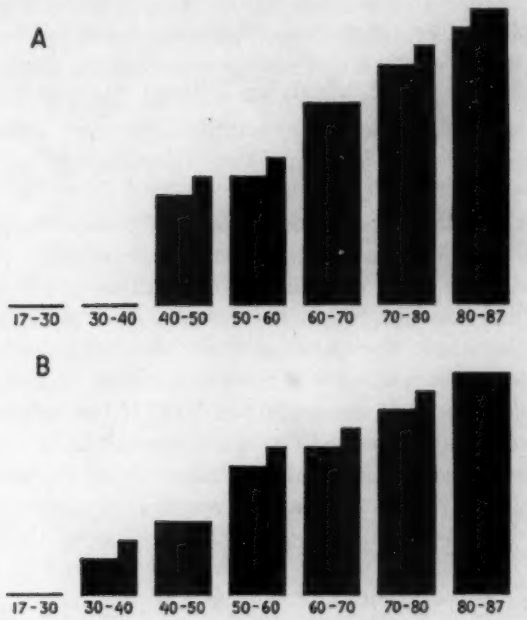

Fig. 1.—Diagram showing the increases in calcification of cartilage with age: *A*, in men; *B*, in women. The numbers represent decades of life. Twelve cases were examined for each decade. An area 0.5 cm. square corresponds to a mass of calcium designated in the text as +.

It must also be remembered that gallstones often occur in young persons and that similarly but less seldom calcified adherent pericardium is found after pericarditis and juvenile arteriosclerosis.[22]

Figure 1 shows clearly that the calcification of cartilage in general and of the areas described in particular belongs among the phenomena which are usually regarded as characteristic of senescence. In view

22. Herxheimer, G.: Centralbl. f. allg. Path. u. path. Anat. **33**:111, 1923. Thoma, R.: Lehrbuch der pathologischen Anatomie, Stuttgart, Ferdinand Enke, 1894. Aschoff.[4a]

of the opinion that special diseases would be of significance in regard to the calcium content of the skeleton inasmuch as they cause dissolving out of the calcium, thus making it possible for the cartilage to take up an excess of this substance, an attempt was made to find common factors between all the cases in which deposits of calcium in cartilage were found, but the only common factor was advanced age.

One also reads in the literature that bronchial asthma specially predisposes to calcification of rib cartilage, but as none of the 200 persons in the present series happened to have suffered from this disease, the problem will not be gone into here. There has been received, however, a strong impression that pulmonary tuberculosis plays a significant role in the calcification of tracheal and bronchial cartilage. It is still too early to express a positive opinion on this point as the series contains only about a dozen persons under 35 years of age. As far as friability of the bones is concerned, the parathyroids were examined microscopically in a few cases of marked osteoporosis, but the results were negative.

The microscopic preparations demonstrate excellently the way in which calcium is deposited in the tissues and in what quantity. Not only do these slides show the calcium in the cases in which the deposits were found macroscopically but they show rather plentiful amounts in many cases in which the cartilage had been regarded as free from calcium. If one counts cases in which these milder deposits were shown, the number of "positive" cases reaches 100 (if one selects 50 years of age as normal). Then, of course, the organism as a whole begins to alter with age. As mentioned, this calcification of the cartilages of the respiratory tract of a more marked type begins seriously at from 45 to 55 years of age in both sexes. Calcification of the rib cartilages probably sets in a little earlier and is more marked in men. In the present series 70 per cent of 2 plus and 3 plus lesions were in men.

Situation of Calcium Depots.—Two large groups of cartilages have been examined in this respect: (1) the cartilages of the ribs and (2) the cartilages of the trachea and bronchi.

As regards the rib cartilages, the site of predilection for the deposits lies in the caudal ribs. Aside from the calcification and conversion into bone of the cartilage of the first rib, which occurs at a relatively early age, the cartilages down to the fourth and fifth rib often show remarkably few lesions compared with those to be found in the cartilages from the sixth to the ninth, the lesions here taking the form of clumps and strands of calcium. These masses of calcium are often so exaggerated in size that they can be picked out of the cartilage with the point of a knife. A horizontal section often shows them extending several centimeters through the cartilage and having a thickness of several millimeters. Cutting through such a cartilage gives the sensation of a soft

mass impregnated with small stones. In connection with the calcium deposits one not infrequently finds areas of appositional new bone formation.[23] Such areas appear as small cavities filled with bone marrow and surrounded by strands of young bone, which also traverse the marrow cavity. Thus there is a clear difference between new bone formation in these areas and that seen in the larynx. There the bone forms a dry, gray structure of calcium-filled lamellae, and the cavities contain little or no bone marrow. This may depend on the great difference in age at which such bone formation occurs, for it often begins in the larynx as early as from 20 to 30 years of age [24] and in the rib cartilages scarcely before the age of 60.

The calcification in the trachea and bronchi shows a marked difference between the sexes as far as its situation is concerned. In men the deposits extend from the bronchi up to the cricoid cartilage, thus corresponding well with the recognized circumstance that the male larynx is calcified and converted to bone much oftener than is the female larynx. This can be observed not merely when there is complete calcification of the cartilage of the respiratory tract but also and perhaps more strikingly when only the large tracheal cartilages are filled with calcium and the bronchi are at least macroscopically free.

On the other hand, in women it is a rule to find the bronchial cartilages, from the largest to the smallest the site of calcium deposits, while the trachea is involved in only about every tenth case.

It was mentioned that in 169 of 200 cases deposits of calcium were demonstrated. It may seem peculiar that in only 31 cases were the cartilages free from such changes, but in the tabulation, under the head-

Calcified Cartilage		Calcified Tracheal and Bronchial Cartilage	
Men	Women	Men	Women
84	85	58	78

ing "Calcified Cartilage," has been listed each case in which calcium was shown in abnormal amounts, regardless of its site or amount.

There is a marked difference in the sexes in regard to the site of the deposits. In men the rib cartilages are the usual site of calcification while in women the cartilages of the trachea and bronchi are involved to a much greater extent. The accompanying tabulation shows that 2 per cent more women than men had this alteration in the cartilages of the trachea and bronchi. This distinction becomes still clearer if one computes for each sex the number of cases in which calcium could be demonstrated at autopsy, that is to say, the cases in which the calcification was graded 2 and 3 plus: men, 25; women, 53.

23. (*a*) Pommer, G.: Ztschr. f. Anat. u. Entwcklngsgesch. **75**:382, 1924-1925. (*b*) Weidenreich, F.: ibid. **69**:382 and 558, 1923.

24. Pascher, A.: Virchows Arch. f. path. Anat. **246**:198, 1923. Friedmann.[2]

Different Types of Calcification.—The exact mechanism of the calcifications of cartilage and also of the calcification in normal formation of bone is not yet clear. Under the microscope one can, however, follow the gradual invasion of the tissue by calcium. On the whole, there are three different ways in which the calcium settles in the cartilage: (1) as a diffuse distribution of small kernels; (2) like a capsule around

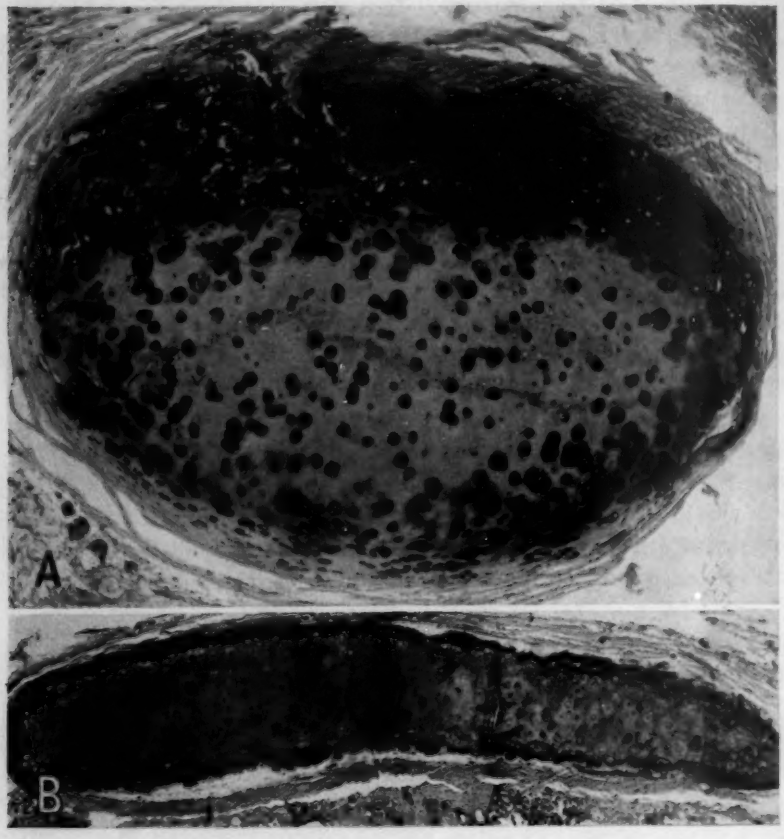

Fig. 2.—Exoplasmatic calcification: *A* exemplifies the earlier stage; × 125. The upper part of the cartilage is surrounded by a beam of calcium. *B* shows the termination of the process; × 70. A crust lies round the cartilage. The tissue shown in *A* was from a woman 47 years of age; that shown in *B* was from a woman of 77 years.

the dying cartilage; (3) in the form of strands which traverse the interstices between the endoplasmic areas, out in the exoplasm. In cases belonging to group 2 there are almost always calcium kernels also in the center, which indicates that this stage is a later one.

These kernels of calcium appear either around or within preformed parts of the hyaline cartilage, "cartilage cells" or endoplasmic areas (Hansen [1]). Several authors have described the beginning of this process as follows: In the layer of chondrin which is separated from the endoplasmic areas and thus seems to form capsules around them the calcium salts are deposited. Gradually a partial or complete shell is formed around the endoplasmic area in question and if the process continues the picture is that seen in figure 2 *A*. The isolation of the endoplasmic area leads to its necrosis and later to calcification of the type which occurs in many other necrotic tissues in the body. The result is a calcified sphere lying embedded in exoplasm.

None of the cartilage escapes the deposition of heterogeneous material, neither the endoplasm nor the exoplasm. Without doubt, this calcifying process implies a marked decrease in the metabolism of the cartilage and thus in its vitality.

Type 1. As the endoplasmic areas chiefly represent metabolic centers (and thus the most actively living parts of the cartilage), their destruction means that irreplaceable damage has been done to the entire cartilage. One can see microscopically that after the endoplasmic areas are injured, the exoplasm loses its stainability and disintegrates in the form of threads (asbestous degeneration) or otherwise.

Type 2. Here the calcification takes the form of threads or lamellae situated in the periphery of the cartilage, which, crowding the chondrin or the endoplasmic areas to the side, join other strands to form a shell around the still living tissues. One can imagine that the blood supply to the cartilage becomes more and more unsatisfactory with age and that as a result the endoplasmic areas undergo necrosis and secondary calcification. In connection with these changes follow decreased vitality and calcification of the exoplasm.

Type 3. One can observe "exoplasmic infiltration" in every case in which definite calcification is shown, either of the diffuse type, with small kernels, or of the type in which a calcified capsule is formed around the cartilage. It seems as if this is the most usual way in which calcium appears, as is suggested also by the fact that this process was observed and described long ago.[25] This diffuse infiltration seems, however, to differ somewhat from the previously mentioned more solid calcium deposits in that it appears at an earlier age and, in my opinion, especially in connection with some wasting disease, particularly pulmonary tuberculosis. This type of diffuse calcification which increases the resistance to a knife has been described under the heading "Technic." Both types 2 and 3 represent calcification of the exoplasm, but the dis-

25. Orth.[3a] Hansen.[1]

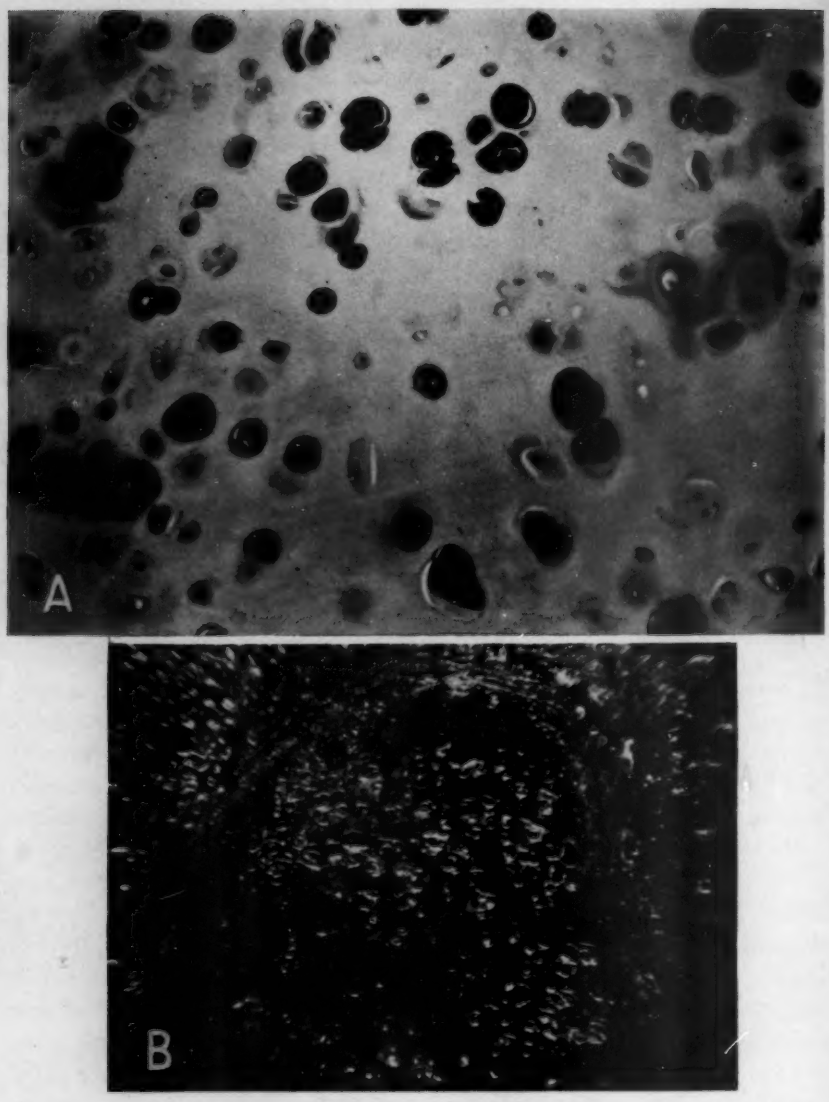

Fig. 3.—*A*, detail of endoplasmatic calcification; × 350. The completely calcified endoplasmatic areas stand out sharply against the exoplasm. This tissue was from a woman of 74 years. *B*, incinerated cartilage under dark field illumination, showing endoplasmatic calcification, in which a shell forms around the inner parts, and exoplasmatic calcification, in which the calcium forms rings round the endoplasmatic areas or covers them wholly.

tinction in the microscopic picture as well as in the effect is so clear that a division into two types is fully justified.

In these different ways calcification took place in the cases in which deposits of calcium in cartilage were definitely present. As mentioned, the cases in which the cartilage was macroscopically negative for calcium were also examined histologically, and 3 of the 12 showed undoubted signs of calcification. The quantities of calcium in these cases were considerably lower than in the aforementioned cases, as they could not be demonstrated macroscopically. The type of calcification corresponds to that described as type 3.

Effect of Calcification.—The effect of calcification on a cartilage ring is that the vitality and functional activity are decreased or wholly destroyed. The calcium kernels that appear in the endoplasmic areas, like the capsule that is formed around the cartilage, exclude the cartilage more and more from sufficient oxygen and nourishment. The calcium shell prevents the inflow of blood in a mechanical way, and the endoplasmic calcification, which implies destruction of the metabolic centers in the cartilage, has the same effect as disconnection of the digestive canal would have in man. Different parts of the cartilaginous ring undergo degeneration or necrosis, and the once so pliable cartilage is converted into a shrunken, uneven, stiff, hard piece of bronchus. The outer shape of the cartilage ring is altered in a similar way. One autopsy report from the Caroline Institute reads:

> The bronchi are wide open, with stiff curves, and the calcium masses in the cartilaginous rings grate against the knife. The lumen is uneven, because the bronchial framework is shrunken and infiltrated with calcium kernels which project into the lumen.

Such a disturbance of the normal anatomy of the bronchi is naturally followed by a decrease in functional activity. Pulmonary ventilation does not suffer particularly, but the resistance to dust and microorganisms is reduced. This clearly makes possible a greater number of infections and pneumonokoniosis. Small particles are easily aspirated into the deeper portions of the lungs through these stiff upright tubes, and behind the transverse pockets the bacteria can easily settle and produce inflammatory lesions in the mucous membrane of the bronchi and even in the lungs. In addition, the expectoration of secretion and inflammatory exudate is made more difficult, and thus the danger of pulmonary disease increases. The calcification of the bronchi does not play the main role in the origin of these diseases, which are often only the final phase in the process, but the calcification still seems to have some significance in the process.

Calcium Deficiency in the Skeleton and Calcium Deposits in Cartilage.—Senile osteoporosis arises through several contributing processes: halisteresis, resorption by osteoclasts and perforation canals.[26] A deficiency of calcium develops in the bone, and big masses of calcium salts in solution are given up to the blood over long periods and deposited in places suitable for one reason or another. This is the theory of Virchow and his pupils as to the nature of calcium metastases. They mean, in short, that a faulty site for the calcium is the cause of the disease. On the one hand, the skeleton is poor in calcium owing to carcinosis, large cysts or other unknown and uncertain causes and, on the other hand, there is an excess of calcium in the other body tissues. Supported by this theory, one is thus free to regard these calcium metastases as representing a higher, more pathologic degree of calcification than the calcification of cartilage described earlier in this paper. Or should one consider the latter as calcium metastasis of a milder grade?

In this way one can well explain the common coincidence of osteoporosis and large calcium depots in the cartilage. This explanation also fits in well with the circumstance that friability of bone is much more common in women than in men and that the higher grades of calcification, especially of calcification of tracheal and bronchial cartilage, are found in women.

Calcified Cartilage		Osteoporosis	
Males	Females	Males	Females
84	85	8	55

The mechanism of the regulation of calcium metabolism in the body is still unknown. The difference between the sexes points in the direction of the internal secretion, but in the present material none but senile lesions could be demonstrated in the uterus and ovaries. Pregnancy must play a prominent role, but since no exact data are available as to the relation between gravidity and parturition, on one hand, and this excess of calcium, on the other—just the opposite to puerperal friability of bone—the question must remain open for further investigation.

Perhaps these conditions can be explained by Häggqvist's [27] theory as to the capacity of protoplasm to hold calcium in solution or release it according to need.

Calcified Vessels (Advanced Arteriosclerosis) and Calcified Cartilage.—In every one of the 51 cases of marked calcification of the

26. von Recklinghausen, F.: Untersuchungen über Rachitis und Osteomalacie, Jena, Gustav Fischer, 1910. Pommer.[23a]

27. Broman, I., and Häggqvist, G.: Text-Book of Histology, Stockholm, Albert Bonnier, 1928.

vessels (advanced arteriosclerosis) there was also shown calcification of cartilage. There thus exists a striking parallelism between calcification of cartilage and calcification of the atheromatous areas in the vessels. Even if it is not justifiable to identify the two processes, it is clear that in both some destructive force has been at work to prepare for the subsequent calcifications. It should also be mentioned here that friability of bone was observed in about 40 per cent of the cases in which the vessels were calcified.

Chemical Analysis.—Specimens of rib, tracheal and bronchial cartilage were ashed and analyzed. As in normal bone, the calcium was present in the form of phosphates and carbonates. There were also traces of magnesium salts. All of this corresponds well with the view that the calcium deposited in the cartilage comes chiefly from the calcium supply in the bones.[28]

SUMMARY

Rib, tracheal and bronchial cartilage taken from older persons was examined with reference to the occurrence of calcium. Calcification is common from 50 years of age on. In only 41 of the 200 cases was the cartilage macroscopically free from calcium. In 10 of the 41 cases the persons from whom the cartilage was obtained were less than 40 years of age and in many of the other cases the cartilage showed microscopic calcium deposits.

In men the rib cartilages are most often involved and preferably those of the fifth to the ninth rib (the cartilage of the first rib is a known exception). Women show a higher degree of calcification in the cartilages of the trachea and bronchi.

In the milder process the calcium appears in the cartilage in the form of a diffuse infiltration of the salts. When the process is more advanced, the calcium forms either kernels or lamellae, which destroy the endoplasm and exoplasm or form capsules of confluent lamellae around the cartilage.

There is often a clear correlation between calcium deficiency (osteoporosis) and calcification. Other anomalies of calcium metabolism are often observed in connection with calcification of cartilage; examples are the formation of gallstones, calcification of the mitral valve and fat-calcium infarction of the kidneys.

No certain cause of the described changes could be demonstrated. This is undoubtedly due to their being partially a phenomenon of the age process, whereby they evade causal investigation.

28. Hofmeister, F.: Ergebn. d. Physiol. **10**:429, 1910. Tanaka, M.: Biochem. Ztschr. **35**:113, 1911; **38**:285, 1912.

TISSUE REACTIONS TO NATURAL OILS AND FRACTIONS THEREOF

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The mammalian body, although rich in cells and permeated by vascular and lymphatic channels, is composed principally of intercellular materials which are in a more or less continuous state of flux. These materials form a matrix or a phase which is of variable composition in the several parts of the body. It is known that local changes in the intercellular phase may alter the form and activity of the cells with which the phase is in contact. It is also generally recognized that changes in the cells may alter the nature of the regional phase. Most substances, beneficial or deleterious, gain access to the cells only after traversing the intercellular medium. Conversely, the metabolites of cells are usually constrained to pass through this medium before they reach the various excretory channels.

Despite the probable importance of the mechanisms which are operative in the intercellular region, knowledge concerning them is limited. Intracellular processes have not been distinguished clearly from those which may be effective only in the intercellular phase. This lack of distinction is to some extent a result of unwarranted inductions from experiences with extracts of macerated tissues isolated from the constraints of the tissue environment of the intact animal.

It seemed reasonable to believe that some of the local intercellular mechanisms might be exposed by an artificial introduction into the intercellular phase of certain classes of organic compounds. Furthermore, it seemed desirable initially, in view of the excellent work of Sabin [1] and Anderson [2] on the lipoids of the tubercle bacillus, to employ natural unreactive and reactive compounds which would remain localized in the region of injection for a long period of time. This plan was followed, and the descriptive studies to be reported here are concerned first with local reactions accompanying the presence of cod liver oil and olive oil in the intercellular medium of the subcutaneous tissues, second with the local reactions to convenient fractions of these oils and third with the transformations which occur in the physical character of the oils and fractions thereof during a period of residence in the intercellular environment.

From the Department of Pathology, Harvard University Medical School.

1. Sabin, F. R.: Physiol. Rev. **12**:141, 1932.
2. Anderson, R. J.: Physiol. Rev. **12**:166, 1932.

MATERIALS AND METHODS

Several preliminary experiments were required for standardization with respect to an appropriate animal, a site of injection, a dosage and an interval of time sufficiently brief to expedite experiments and sufficiently long to permit the responses to reach a maximum from a histologic standpoint. A choice of animals was made among guinea pigs, rats and rabbits. Olive oil and cod liver oil were injected subcutaneously, intramuscularly and intraperitoneally in doses of 0.5, 0.25, 0.1 and 0.05 cc. Old and young animals were used. The tissues were examined at intervals of one, two and three weeks after the injection of each oil. The following standards were chosen as most satisfactory: young guinea pigs weighing from 250 to 350 Gm.; subcutaneous injection; a dose of 0.1 cc., and an interval of time of two weeks. These were held constant in almost all experiments with compounds liquid at 37.5 C. With a few of the highly destructive compounds it was necessary to reduce the dose to as little as 0.025 cc. When materials were used which are solid below 37.5 C., amounts varying from 10 to 100 mg. dissolved or suspended in ether or in Tyrode's solution were injected. The amount of solid which was used within the prescribed limits depended on the tolerance of the tissues.

All injections were made with a 1 cc. graduated tuberculin syringe. The materials were introduced into each of the quadrants of the abdominal wall in the region of the intersection of the midtransverse and median lines. The results which are recorded here are derived from a histologic study of the reaction of the tissues to each oil or fraction thereof in four sites of injection in separate animals and in different abdominal quadrants. Records of the progress of the lesions were made every three or four days. The animals were killed at the end of two weeks. Sections of the abdominal wall, including the zones of reaction, were removed and fixed in solutions of formaldehyde U. S. P. (1:10) for from twenty-four to forty-eight hours. The blocks were then cut in slices from 2 to 3 mm. thick, and those including the zone of reaction were dehydrated in graded alcohols, embedded in paraffin and sectioned. The sections were stained with hematoxylin and eosin for histologic study and with phenolized fuchsin by the Ziehl-Neelsen method for the demonstration of acid-fast material.

Medicinal cod liver oil and olive oil of a high grade were used. In the preparation of the various fractions for study, the crude oils were treated as follows: The free fatty acids (olive oil, 1.6 per cent, and cod liver oil, 1.2 per cent) were removed from small samples of each oil in ether by repeated shaking of the ethereal solution with dilute aqueous potassium hydroxide. After the alkali was washed out, the samples were dried in vacuo and, as with other fractions to be described, were stored in an atmosphere of carbon dioxide. Titrations of each oil several days after extraction of the free acids disclosed no free acid. In the preparation of the major fractions of each oil, 500 Gm. was hydrolyzed with alcoholic potassium hydroxide, and the nonsaponifiable ether-soluble materials were extracted. The total nonsaponifiable fraction thus obtained was divided into a portion soluble in about 20 parts of absolute methyl alcohol and a residue soluble in purified petroleum benzene (petroleum ether; boiling point from 40 to 60 C.). The solution containing the products of hydrolysis freed from the nonsaponifiable fraction was acidified with sulfuric acid, and the fatty acids were extracted with ether. The ether extract was dried with sodium sulfate and then concentrated by distilling off the ether and subsequently drying in vacuo. A small portion of the total acid fraction in each instance was preserved. The remaining total acid fraction of each oil was separated by the usual lead salt–ether

method into fractions largely composed of saturated and unsaturated acids. A sample of each of these fractions was preserved. The remainders were converted to methyl esters by the use of methyl sulfate. After storage of samples of the total esters of saturated and unsaturated acids, the remainders were fractionally distilled in high vacuum (about 1 mm. of mercury). One saturated and one unsaturated fraction of the esters of olive oil acids were obtained, only a small residue remaining in each instance. Three fractions of methyl esters of saturated acids of cod liver oil were obtained. In one distillation six fractions and in another seven fractions of the methyl esters of unsaturated acids of cod liver oil were isolated.

The iodine numbers of the several mixtures of compounds as determined by the Wys [3] method are given in the table.

After removal of the ether-soluble nonsaponifiable substances and acids, the residue of the products of hydrolysis was concentrated by

Material	Boiling Point of Methyl Esters in High Vacuum, C.	Iodine Number
Olive oil	.....	83.1
Saturated acid fraction of olive oil	145-168	2.9
Unsaturated acid fraction of olive oil	152-160	78.0
Nonsaponifiable fraction of olive oil	.....	308.2
Cod liver oil	.....	140.4
Saturated acid fraction of cod liver oil	144-181	10.6
Unsaturated acid fraction of cod liver oil	140-208	157.4
Unsaturated acid fraction of cod liver oil	145-155	101.4
	155-161	112.8
	161-172	123.4
	172-180	162.1
	180-188	196.7
	188-196	239.8
	196-204	262.0
Unsaturated acid fraction of cod liver oil	155-165	106.2
	165-175	130.5
	175-180	170.0
	180-190	204.5
	190-195	247.7
	195-205	268.1
Nonsaponifiable fraction of cod liver oil	.....	324.6

distilling off the alcohol and water. The inorganic salts were largely crystallized from the concentrate by the addition of 10 volumes of absolute methyl alcohol at —20 C. After distilling off the alcohol, the residue, composed largely of glycerol, in each instance was preserved as the glycerol fraction.

EXPERIMENTAL RESULTS

The experimental data enable one to make correlations between groups of related compounds and the types of response which accompany their presence in the intercellular environment of the subcutaneous tissues. Comparisons and contrasts will be drawn, with emphasis on the following aspects: first, the magnitude of the tissue response; second, the participation of the epithelium in the reaction; third, the

3. Wys, J. J. A.: Ber. d. deutsch. chem. Gesellsch. **31**:750, 1898.

presence of various types of inflammatory cells in the neighborhood of the residual compounds, and, fourth, the alterations that occur in the properties of certain materials during their residence in the imposed environment.

Magnitude of the Tissue Response.—The term "magnitude of the response" applies to a weighted composite of the degree of injury to the tissues and the degree of the inflammatory reaction. The two natural oils as irritants clearly belong in different categories. The removal of the free acids (from 1 to 2 per cent) from each oil prior to injecting the oil does not materially alter either the extent of injury to the tissues or the degree of the inflammatory reaction which occurs in the presence of the crude oils. Nor is there any morphologic evidence that the nature of the reaction of the tissues to olive oil is altered by adding to the olive oil a small complement (from 1 to 2 per cent) of acids isolated from cod liver oil. The reaction to olive oil is very mild. It reaches a maximum during the first few days and thereafter declines in magnitude. The oil appears to persist unchanged in the tissue spaces, disappearing gradually over a long period of time. Cod liver oil is productive of an injury to the tissues and a cellular reaction of a fairly high degree of intensity. The order of the reaction is low in the first few days; then the response gradually increases so as to reach a maximum during the third week. The oil undergoes definite alterations while in the intercellular phase, and at the end of three weeks little unchanged oil remains at the site of injection.

The glycerol fractions of cod liver oil and olive oil excite little reaction. The response to the fraction isolated from the products of hydrolysis of cod liver oil exceeds that to the corresponding fraction from olive oil. The addition of glycerol fractions or of pure glycerol to equal parts of acids, esters or potassium salts of acids is effective only in increasing the magnitude of the reaction to the acids, esters or salts of acids. These experiments yield no evidence of a local synthesis of adipose tissue from the injected materials or of any significant influence of glycerol on the types of cellular reaction.

The order of the reaction to the nonsaponifiable components of each oil is in a higher category than that to all other compounds except the highly unsaturated acids. It is necessary to reduce the dosage to prevent ulceration of the skin. The destructive elements are chiefly restricted to the alcohol-soluble compounds in each nonsaponifiable fraction. (It may be pointed out here that the percentage of nonsaponifiable material in each oil is approximately the same as the percentage of the free fatty acids.)

The reactions of the greatest magnitude, in general, follow injections of acids. The total acid fraction of cod liver oil is more destructive and excites a more pronounced inflammatory reaction than the cor-

responding acid fraction obtained from the products of hydrolysis of olive oil. The total fraction of the unsaturated acids of cod liver oil produces responses of a higher order than the total fraction of saturated acids of cod liver oil or of olive oil or that of the unsaturated acids of olive oil.

There is good evidence that the order of response to the various fractions of methyl esters of acids is but slightly lower than that which follows injection of the unesterified acids. The magnitude of the reaction varies with the nature of the acyl radicle of the ester. The methyl esters of the total fraction of unsaturated acids obtained from cod liver oil excite reactions of a much higher order than the esters of the corresponding fraction of acids derived from olive oil. The tissue injury and the tissue reaction produced by the presence of any of the several fractions of methyl esters of unsaturated acids of cod liver oil increase with the boiling point of the fraction. The elevation of the boiling point, in turn, is principally a function of the increasing length of the carbon chain of the acids in question and therefore becomes also a function of the increasing average unsaturation of the fraction.

Participation of the Epithelium in the Response.—The behavior of the stratified squamous epithelium and of the epithelium of the cutaneous appendages is particularly interesting. One frequent proliferative reaction will be described: The cellular activity occasionally results in partial epithelization of the cysts which are formed as the result of fibrous encapsulation of the residual material and exudate at the site of injection. This participation of the epithelium in the defense mechanism occurs most frequently at the edges of lesions produced by the methyl esters of the highly unsaturated acids of cod liver oil. The epithelium, stratified squamous in type, appears on the side of the lesion which is adjacent to the epidermis and extends for variable distances so as to encyst the foreign material. No cysts entirely lined with squamous epithelium are encountered, but the encapsulation is often surprisingly extensive. The result is a morphologic structure which is similar to the so-called dermoid or sebaceous cyst. The origin of the epithelium is not understood in all instances. Sometimes this lining arises from the margin of a superficial erosion. In other instances, if such erosions are present, they are not detected grossly and are not found in the sections. A papillary downgrowth of columns of epithelial cells is common. These columns of cells arise not only from the malpighian layer of the epidermis but also from the basal cells of hair follicles. They often form epithelial pearls composed of well differentiated squamous cells. The continuity of this proliferating epithelium with the stratified squamous epithelium that lines the cysts is demonstrable.

Participation of Inflammatory Cells in the Response.—The accumulation of neutrophils in a zone of reaction can be correlated as a rule

with the magnitude of the response, as described for the various materials.

Eosinophils, the role of which in normal or pathologic states is unknown, are so distributed among the reactions that special attention may be directed to them. They are rare in the reactions to crude olive oil, acid-free olive oil and olive oil to which a small complement of acids of cod liver oil has been added. Several groups of eosinophils are invariably found in the zone of response to crude cod liver oil and in that to acid-free cod liver oil. Fractionation of the oils discloses that the unsaturated acids and the esters of unsaturated acids create local conditions which predispose to infiltration by these cells and that they are often conspicuously numerous in the regions of reaction to the high-boiling unsaturated ester fractions of acids isolated from cod liver oil. The glycerol, nonsaponifiable and saturated fractions are not effective in stimulating this type of cellular infiltration.

Macrophages for the present purposes may be roughly subdivided into three general morphologic types: first, those with homogeneous cytoplasm; second, those with large vacuoles in the cytoplasm, and, third, those with delicately reticulated foamy cytoplasm. The three types are present in the zones of reaction to the oils. In the zones of response to olive oil the cells are few, and most of them have homogeneous cytoplasm. In the zones of reaction to cod liver oil macrophages increase numerically with time. Most of them have homogeneous cytoplasm, others are vacuolated, and a few contain globules and granules of insoluble material. Foamy macrophages are found only after careful search. Macrophages with relatively homogeneous cytoplasm are numerous in the reactions to the nonsaponifiable, unsaturated acid and unsaturated ester fractions. Macrophages with delicately reticulated foamy cytoplasm are predominant in reactions to the methyl esters of saturated acids. Here, they occur in large numbers and form a distinctive feature of the histologic picture. The addition of glycerol to acids, esters or potassium salts of acids does not alter the number or the nature of the macrophages.

The multinucleated giant cells which are encountered in any particular reaction have in general the same cytoplasmic characteristics as the macrophages. The formation of these cells seems to be determined by conditions which will be mentioned in the subsequent paragraphs.

A few small crescentic cells with three or four nuclei in each are often closely applied to the margins of globules of olive oil. The giant cells which occur in the zones of reaction to crude and to acid-free cod liver oil increase numerically with the time of residence of the oil in the intercellular phase. These multinucleated cells attain a large size. They are principally applied to the relatively insoluble membranes and filaments of the altered oil.

Giant cells are fairly numerous in the zones of reaction to the total acids of olive oil. The stimulus to their formation seems to be restricted principally to the saturated fraction of the acids. The large foamy multinucleated cells which occur here do not appear in the reaction to the unsaturated fraction. The total acid fraction obtained from cod liver oil excites formation of numerous giant cells. In contrast to the experience with acids of olive oil, the numerical distribution of the multinucleated cells between the reactions to the saturated and the unsaturated acid fractions of cod liver oil is about equal. The cells which are formed in the response to the saturated fractions of each oil are characteristically applied to the boundaries of crystal clefts. The giant cells which are found in the reactions to the unsaturated acid fractions are in almost every instance applied to the margins of relatively insoluble amorphous material, which seems to arise through some transformation of the unsaturated compounds during their residence in the intercellular phase.

The results obtained in the study of the acid fractions which are solid at the temperature of the body are difficult to analyze. For this reason the methyl esters of these acids were prepared and administered. The esters are liquid at the body temperature. Fortunately, they are readily hydrolyzed in the intercellular phase. This permits a direct comparison of the effect of saturated acids with that of unsaturated acids. In the instance of liquid esters of saturated acid fractions, the chief detectable product of hydrolysis is an acid which is deposited gradually as a crystalline material. There is, therefore, a qualitative similarity between the reactions produced by the methyl esters and the reactions produced by the corresponding unesterified acids. Macrophages and multinucleated giant cells with delicately reticulated foamy cytoplasm occur in large numbers in the zones of reaction to the saturated methyl esters. The giant cells are applied principally to the margins of crystal clefts.

The methyl esters of the unsaturated acid fractions as a rule excite reactions which are similar to those of the unesterified acids. This indicates that these esters are hydrolyzed at a rate comparable to that of the liquid methyl esters of the saturated acids. The methyl esters of unsaturated acids obtained from olive oil stimulate no significant formation of giant cells. The methyl esters of unsaturated acids of cod liver oil often induce reactions in which giant cells are numerous. The increase in the number of giant cells presents a positive correlation with three characteristics of the fractions of methyl esters of the unsaturated acids of cod liver oil: first, an increase in the boiling point of the fraction; second, an increase in the average unsaturation of the compounds in the fraction, and, third, an increase in the amount of relatively insoluble amorphous material which is formed in the zone of reaction to the fraction.

The conclusion is reached that the only important stimulus to formation of giant cells in the zone of reaction to saturated compounds is the presence of solid crystalline materials in the tissues and that the only important stimulus to formation of giant cells in the zones of reaction to the unsaturated compounds is the presence of relatively highly insoluble amorphous materials which arise through some transformation of the unsaturated compounds during their residence in the intercellular phase.

Transformations of Compounds in the Intercellular Phase.—The study of the reactions to the various mixtures of compounds indicates that some of the materials which are injected do not retain their character while in the intercellular phase. It is clear that hydrolysis of esters is responsible for one transformation. Other transformations are not so easily explained. There is good evidence that the liquid methyl esters of unsaturated and of saturated acids are readily hydrolyzed in the intercellular medium. Two reasons for arriving at this conclusion have been mentioned: First, there is a deposition of crystalline acids at the site of injection of liquid methyl esters of saturated acids; second, the methyl esters stimulate a tissue response which is similar to that occurring in the regions of reaction to corresponding unesterified acids. Under similar conditions in the intercellular phase, olive oil, which is composed primarily of glycerides, as are the depot fats of the body, is not appreciably hydrolyzed. The study of the reactions to cod liver oil, which is principally composed of glycerides, although not clearly disclosing any evidence in favor of hydrolysis, shows that a part of the oil is transformed into an amorphous semisolid material which is often insoluble in water, absolute alcohol and xylene. This alteration is similar to that which has been described by Pinkerton [4] as occurring in several oils during their residence in the air passages of the lungs. This transformation in the intercellular phase of the subcutaneous tissues, although not impressive during the first week, progresses rapidly during the second and third weeks. The material appears first in the form of a membrane at the zone of contact of the oil with the tissues. The membrane is usually homogeneous. Occasionally it presents a fibrillar appearance, due perhaps to included strands of fibrin. In time the membrane breaks up into shreds, filaments and granules. Small globules of oil often maintain an intact boundary of this material, and the boundary increases in thickness until the globule is transformed wholly into an insoluble substance. The small shreds and globules are frequently incorporated in the cytoplasm of macrophages and giant cells. The large shreds, filaments and globules are partly or wholly encapsulated by macrophages and giant cells.

4. Pinkerton, H.: Arch. Path. **5**:380, 1928.

Some of the insoluble material, usually slightly yellowish, gradually assumes the property of retaining the phenolized fuchsin, a property which is possessed by the acid-fast micro-organisms. The acid-fast property is first detected at the margins of the insoluble material and in time seems to spread centrally. The degree of retention of the stain varies but in general it increases with time and with the extent of contact of the oil with the tissues. The delicate filaments which are so numerous after two or three weeks' residence of the oil in the tissues are as brilliantly stained with phenolized fuchsin by the Ziehl-Neelsen method as are acid-fast bacilli, such as the tubercle bacillus and the leprosy bacillus. The material is as abundant in the zones of reaction to acid-free cod liver oil as in the regions of response to crude cod liver oil. Commercial olive oil, acid-free olive oil and olive oil plus a small complement of acids derived from cod liver oil yield no insoluble material or acid-fast material. The glycerol fractions and the highly unsaturated nonsaponifiable fractions of the oils contain no compounds which undergo this transformation. The total acid and the unsaturated acid fractions of olive oil yield a few granules of acid-fast material in macrophages. The conversion of the acid fractions of olive oil into esters and subsequent distillation of the esters result apparently in a loss of the small amount of substance or substances from which the acid-fast insoluble material is formed. There is a fairly large amount of the substance which retains the phenolized fuchsin in the zones of injection of the total acids of cod liver oil. This material is found to be derived exclusively from the compounds in the unsaturated fraction of the total acids.

The methyl esters of the saturated acids derived from the oils do not yield any insoluble or acid-fast material.

The methyl esters of the total unsaturated acids of cod liver oil give rise to formation of an abundance of insoluble material, a part of which is acid-fast. The amount of this substance which is formed in the zone of injection of any of the various fractions of these esters is a function of the boiling point of the ester employed. The boiling point, in turn, of esters of acids derived from cod liver oil, is principally a function of the length of the carbon chains of the acids. Inasmuch as the cod liver oil acids with long carbon chains have the highest degree of unsaturation, the increase in the amount of insoluble material and acid-fast material in the zones of reaction to the methyl esters of these unsaturated acids is positively correlated with an increase in the average unsaturation of the compounds in the fractions employed. This is clearly shown by the iodine numbers in the table.

Further progress in the study, first, of the significance of various patterns of cellular reaction as related to the chemical structure of compounds used to produce the reactions, second, of the factors which govern the hydrolysis of esters in the intercellular phase and, third, of

the mechanism involved in the transformation of certain liquid unsaturated carbon compounds during their residence in the intercellular environment into amorphous semisolid homogeneous materials may be made by employing purified chemical compounds.

SUMMARY

Olive oil persists in an unchanged state during a period of three weeks' residence in the intercellular medium of the subcutaneous tissues. During the same interval of time cod liver oil is partly transformed into an amorphous homogeneous semisolid material. A part of this material is insoluble in water, alcohol and xylene. Some of the insoluble substance is acid-fast.

The study of the various fractions of the hydrolyzed oils and the methyl esters of the acid products of hydrolysis shows that the amorphous semisolid material is formed in the intercellular region only in the presence of unsaturated fatty acids or methyl esters of unsaturated fatty acids. Furthermore, an increase in the amount of this material is positively correlated with an increase in the average unsaturation of the fractions employed.

In addition to the formation of the amorphous substance, there are three features which characterize the reactions to the methyl esters of the unsaturated acids of cod liver oil. First, the increase in the intensity of the inflammatory response is associated with an increase in the average unsaturation of the fraction introduced into the tissue spaces. Second, an infiltration with eosinophils is prominent in the zones of reaction to several of the unsaturated fractions in contrast with the relative infrequence of these cells in the zones of reaction to saturated compounds. Third, the numerical distribution of multinucleated giant cells in the regions of response to the unsaturated liquid compounds during their residence in the tissue spaces indicates that the amorphous semisolid material derived from these compounds is the only effective stimulus to giant cell formation.

The liquid methyl esters of the saturated acids employed in these experiments, in contrast with the glyceryl esters, are rapidly hydrolyzed in the intercellular environment. The chief detectable product of hydrolysis is an acid which has crystallized out in the zone of reaction. The numerous giant cells which form are applied principally to clefts from which the crystals of the acids have been dissolved during the preparation of the sections. These cells, as well as the macrophages, have delicately reticulated foamy cytoplasm. This cytologic pattern is a pronounced characteristic of the tissue reactions to the liquid methyl esters of saturated acids and distinguishes them sharply from the reactions to the liquid methyl esters of unsaturated acids isolated from the products of hydrolysis of cod liver oil and olive oil.

EFFECT OF A PRIMARY PULMONARY TUBERCULOUS LESION (BCG) ON EXPERIMENTAL PULMONARY TUBERCULOSIS IN RABBITS

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In previous experiments on the significance of allergy and resistance in experimental tuberculosis the following observations were made[1]: 1. A fair degree of resistance against virulent strains could be obtained by giving animals injections of living or heat-killed BCG intravenously or subcutaneously, with or without allergy. 2. The allergic state hastened the inflammatory reaction of tissue at the point of a secondary infection. 3. An experimental primary lesion (BCG) in the lung of a rabbit produced resistance against local and generalized infection following a subcutaneous inoculation of 0.01 mg. of a virulent bovine strain (Ravenel). In normal animals with a similar subcutaneous injection of an equal amount of the Ravenel bovine strain extensive local and generalized tuberculosis followed. 4. Allergy due to local infections outside the lung as well as within the lung had little deleterious effect but carried with it much protection.

Whether a primary pulmonary tuberculous infection should be looked on as a protective mechanism primarily or as a state carrying with it something which predisposes to more extensive tuberculosis is still a question debated by students of tuberculosis. The literature on this controversy has been considered in a previous paper and will not be taken up in this article.

The purpose of the experiments reported in the present article was to determine the effect of primary pulmonary tuberculosis (BCG) in the left lung on the progress of tuberculosis in the right lung, produced by injecting 0.01 mg. of a virulent strain directly into the lung through the pleural wall and cavity.

MATERIALS AND METHODS

Rabbits weighing about 4 pounds (1.8 Kg.) were the experimental animals. The strain of BCG used for producing the primary nodule in the left lung was one which had been cultured on beef infusion–glycerin agar for several years

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1. Clawson, B. J.: Arch. Path. **20**:343, 1935; **22**:99, 1936.

without the addition of any bile. This strain had been injected in large doses into several hundred rabbits without producing progressive tuberculosis. The virulent bovine strain which was injected into the right lung was the C strain of the Henry Phipps Institute, supplied to me by Dr. M. B. Lurie.

The experimental primary pulmonary lesion (BCG) was produced in the left lung by injecting 2 mg. of living BCG through the thoracic wall and pleural cavity into the lower lobe of the left lung. It was previously found that a nodular mass or tubercle could be produced in the lung by this method in about 100 per cent of cases. Pleurisy never developed. The nodule became caseous in the center and was surrounded by a wall of typical tuberculous reaction. There were epithelioid cells, giant cells, macrophages, lymphocytes and fibrous tissue. These experimental primary tubercles always healed after several weeks by forming a scar and in some cases by calcifying. Extension to other organs or to another lung or another lobe of the same lung never occurred. Organisms still showing the acid-fast stain could sometimes be seen after several weeks, but the rule was for a scar to form with the disappearance of the organisms.

These primary tubercles were graded according to size from 1 to 4 plus; 1 plus was recorded when there was only a scar, difficult to see but definite on microscopic examination; 4 plus, when there was a nodular area, usually caseous, about 2 cm. in diameter; 2 or 3 plus, when the lesion was of an intermediate type. The degree of allergy resulting from the BCG lesion in the left lung was determined as in previous experiments by injecting 1 mg. of old tuberculin intradermally three weeks after the inoculation of BCG into the lung. The degree of cutaneous induration or necrosis was observed forty-eight hours after the injection of tuberculin and if positive was recorded as 1, 2, 3 or 4 plus.

The infection in the right lung was produced by injecting 0.01 mg. of a virulent bovine strain (C) into the lower lobe of the right lung, as was done in the left lung with BCG, through the thoracic wall and pleural cavity.

The injection of virulent bacilli into the right lung was given to 32 normal animals and 38 animals with primary BCG tubercles. For convenience of description the two groups are called normal animals and allergic animals, respectively. The animals in both groups were killed for examination at periods after the injection of virulent bacilli ranging from one to one hundred and ten days.

The size of the primary lesion in the left lung and the amount of tuberculosis in the right lung were observed. An involvement including most of the right lung with extension to the left lung or to other organs, as the kidneys, was recorded as 4 plus. The smallest amount which could be detected by gross examination was counted as 1 plus. Grades 2 plus and 3 plus were intermediate.

RESULTS OF EXPERIMENTS

The amounts of tuberculosis present in the right lungs of the normal and allergic animals at different numbers of days after the virulent infection are noted in the table. The grades of allergy present three weeks after the BCG injection and the sizes of the BCG primary lesions in the left lung at the time of autopsy are also recorded for the allergic animals.

Two animals in each group were killed at intervals of one, two, four and seven days after the inoculation of virulent bovine bacilli. No tuberculosis was found in any of the 8 normal animals. Tuber-

culosis of grades 1 to 2 plus was present in the 6 allergic animals at the one, two and four day periods. On the seventh day the 2 allergic animals showed no tuberculosis.

At the ninth, eleventh and fifteenth day periods the normal animals had tuberculosis in their right lungs of grades 1 to 2 plus, while the allergic animals had no tuberculosis at the ninth day and tuberculosis of grade 1 plus at the eleventh day and of the 2 animals killed at the fifteenth day, 1 showed tuberculosis of grade 1 plus and 1 showed no tuberculosis.

On the twenty-eighth day after the injection of virulent bacilli, 8 normal rabbits and 7 allergic rabbits were killed. Tuberculosis of grades 3 to 4 plus was present in all the normal animals except 2 in which it was 2 plus. Of the 7 allergic animals, only 2 had tuberculosis, and in these it was of grade 1 plus only.

Two animals from each group were killed at the thirty-sixth day. The 2 normal animals had tuberculosis of grades 3 and 4 plus. The 2 allergic animals had tuberculosis of grades 1 and 2 plus. Four normal animals at forty-one days had tuberculosis of grades 2, 3, 3 and 4 plus, respectively. Two allergic animals at the same period had tuberculosis of grades 1 and 2 plus. At the forty-eighth day 4 normal animals showed grades of tuberculosis of 2, 3, 3 and 4 plus, respectively, while 3 allergic animals showed, respectively, no tuberculosis, tuberculosis of grade 1 plus and tuberculosis of grade 2 plus. At the fifty-first day 2 normal animals showed tuberculosis of grades 3 and 4 plus. Of 3 allergic animals, 1 had no tuberculosis and 2 tuberculosis of grade 1 plus.

Normal animals were not studied beyond fifty-one days after the injection of virulent bacilli. Ten additional allergic animals were killed, 5 at sixty-one days and 5 at one hundred and ten days after the injection of the virulent strain. Three of the 5 killed at sixty-one days had no tuberculosis, and 2 had grades of 1 and 3 plus. Of the 5 killed at one hundred and ten days, 2 had no tuberculosis and 3 had tuberculosis of grades 1, 2 and 4 plus, respectively.

In the periods of from one to seven days, inclusive, tubercles appeared much earlier in the allergic animals than in the normal animals. At from nine to fifteen days tuberculosis was noted to be progressing in the normal animals and to be retarded in the allergic animals. From twenty-eight to fifty-one days there was extensive tuberculosis in the normal animals, while most of the allergic animals showed retardation or absence of tuberculosis not only at fifty-one days but for as long as one hundred and ten days after virulent tubercle bacilli were injected into their right lungs.

Allergy of grades 1 to 3 plus was detected in all the animals which had received the primary injection of BCG into their left lungs except 4. It is quite probable that these animals were allergic without the allergy being detected, for in all but one a primary lesion was noted.

Primary pulmonary lesions, grades 1 to 3 plus, were present in all of the allergic groups but one. The degree of allergy and the size of the primary pulmonary lesion in the left lung seemed to bear no relation to each other or to the degree of tuberculosis in the right lung.

Degree of Tuberculosis in Normal Animals, on One Hand, and, on the Other, in Allergic Animals with Primary Pulmonary Lesions (BCG) in Their Left Lungs, at Increasing Intervals After Each Had Received an Injection of 0.01 Mg. of Bovine Tubercle Bacilli (C) into the Right Lung, This Injection Being Made in the Allergic Animals Forty Days After an Injection of BCG into the Left Lung

Normal Animals	Days	Tuberculosis in Right Lung	Animals with Primary Lesion	Days	Tuberculosis in Right Lung	Allergy	Primary Lesion
2	1	0, 0	2	1	+, +	++, +	+, +++
2	2	0, 0	2	2	+, ++	++, ++	++, ++
2	4	0, 0	2	4	+, ++	+++, ++	+++, +++
2	7	0, 0	2	7	0, 0	++, +++	++, +++
1	9	+	1	9	0	+++	+++
1	11	+	1	11	+	++	++
2	15	+, ++	2	15	0, +	0, +	+, ++
8	28	++, ++, +++, +++, ++++, ++++, ++++, ++++	7	28	0, 0, 0, 0, 0, +, +	0, ++, 0, ++, +, ++, ++	+, ++, ++, 0, +, ++, ++
2	36	+++, ++++	2	36	+, ++	++, +	++, +
4	41	++, +++, ++++, ++++	2	41	+, ++	+++, ++	+, +
4	48	++, +++, ++++, ++++	3	48	0, +, ++	+++, +++ ++	++, +, +++
2	51	+++, ++++	3	51	0, +, +	+, ++, +++	++, +, +
			5	61	0, 0, 0, +, +++	++, +++, ++, +, +	++, +++, +, ++, +
			5	110	0, 0, +, ++, ++++	+++, +, +, +++, 0	+++, +, +, +++, +

COMMENT

These experiments illustrate the influence of an existing inactive primary pulmonary tuberculous lesion on a subsequent exogenous infection. A quick inflammatory response is noted. This tuberculous reaction, while more pronounced than in normal animals for a few days, is soon retarded and falls much behind the progress of the tuberculous inflammation in normal animals. This retardation or complete suppression is seen for as long as one hundred and ten days after the time of infection.

The protection observed cannot be attributed to allergy alone, for in other experiments it was found that a marked degree of resistance could be produced artificially both locally and generally in the absence of allergy.

The quick inflammatory response, on the other hand, was probably due, to a great extent, to existing allergy, since the local tissue response has been found to be greater in allergic immune animals than in non-allergic immune animals. It appears evident that the allergy which was associated with other mechanisms of defense in these animals can be considered a factor in resistance by hastening the phenomenon of inflammation and bringing the phagocytic cells in contact with the living organisms more quickly. While allergy may without question destroy tissue in some cases to the extent of spreading the infection, it seems, on the other hand, that assistance in resistance may be afforded by the allergic phenomenon in bringing about the tissue defense more quickly.

These experiments lead one to the inference that a retarded or healed primary pulmonary tubercle may be considered in most cases to have a beneficial aspect (resistance, allergy or both) which in the majority of cases far overbalances the deleterious effects of allergy.

SUMMARY

A primary tuberculous pulmonary focus increases the rate of development of a secondary virulent infection for a few days and then retards or completely prevents further development. Healing of lesions already begun appears to follow.

The experiments demonstrate a beneficial factor in retarded or healed primary pulmonary tuberculosis as well as the absence of a marked deleterious factor.

MORPHOLOGIC STUDY OF THE REACTIVITY OF MOUSE SARCOMA 180 TO BACTERIAL FILTRATES

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Gratia and Linz,[1] in their studies of the Shwartzman phenomenon, observed that the intravenous injection of active bacterial filtrates produced hemorrhages in liposarcoma in guinea pigs. Some of the tumors became hemorrhagic and necrotic. Subsequently Shwartzman and Michailovsky [2] obtained hemorrhages in sarcoma 180 of mice and noted that some of the tumors went on to regression. Since then many investigators (Apitz [3]; Duran-Reynals [4]; Shwartzman [5]; Andervont [6]; Eisen [7]; Fogg [8]; Jacobi [9]; Pommerenke [10]; Rusch and Preston [11]; Shear [12]) have concerned themselves with the reaction of transplantable and spontaneous animal tumors to bacterial filtrates in relation to the Shwartzman phenomenon. In the present study, mice bearing sarcoma 180 were given intravenous injections of meningococcus filtrate in doses sufficient to produce partial or complete regression of tumors, and the tumors were studied grossly and microscopically. A small series of comparative studies were also made on carcinoma 11 and 63 of mice and on transplanted dibenzanthracene tumors of mice; also, the response of sarcoma 180 to irradiation was observed in a small group.

From the Laboratories of the Mount Sinai Hospital.
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EXPERIMENTAL PROCEDURE

Sarcoma 180 of mice obtained from the Crocker Institute for Cancer Research, New York, was transplanted into stock mice. The macroscopic and histologic appearances of the tumor were observed in a control group of 26 mice from the third to the thirteenth day of tumor life (table 1). A second group was given a single intravenous injection of 100 rabbit reacting units of bacterial filtrate on the tenth day of tumor life; the filtrate was capable of eliciting the Shwartzman phenomenon in rabbits. A third group of mice bearing tumors from three to twelve days old was given from 20 to 25 rabbit reacting units of filtrate intravenously. The morphologic character of the tumors in the treated animals was studied at various periods. The animals were killed with ether or chloroform, and the tumors were immediately excised and fixed in solution of formaldehyde U. S. P. diluted 20 per cent or in Bouin's fluid. Hematoxylin and eosin staining was practiced as a routine. Bacterial, Van Gieson's connective tissue, Weigert's elastic tissue, silver and Masson trichrome staining were employed in a number of instances.

TABLE 1.—*Group 1. Controls—Untreated Tumor-Bearing Mice*

Animals	Age of Tumor When Animal Was Killed, Days
3	4
2	5
5	6
3	9
7	11
3	12
3	13
26	

GROWTH AND MORPHOLOGIC CHARACTER OF SARCOMA 180

The gross morphologic appearances of the tumor have been previously described and are summarized here briefly for completeness. Shwartzman,[5] in an extensive experience with the tumor, found that it was possible to obtain nearly 100 per cent of takes in healthy stock mice. Within two weeks of tumor life the mouse mortality was from 7 to 8 per cent (Shwartzman [13]); within four weeks approximately 45 per cent of the tumor-bearing mice died; few survived more than six weeks. The animals usually died with large ulcerating local tumor masses. Metastases were not found. The experiences at the Crocker Institute for Cancer Research, based on inoculations of thousands of mice, indicate that spontaneous regression takes place in approximately 1.3 per cent of noninfected and nonulcerated tumors in healthy animals.

At the site of tumor inoculation a nodule is palpable at about the third or fourth day. It gradually expands in size as a freely movable firm subcutaneous mass. On about the eleventh day the tumor begins to ulcerate, forming a central area of scab, bordered by an erythematous indurated area with a peripherally expanding tumor zone. Prior to

13. Shwartzman, G.: Proc. Soc. Exper. Biol. & Med. **32**:1603, 1935.

ulceration, a small central red area is seen within the tumor in about 50 per cent of instances. Now fixation of the tumor to the skin and underlying tissues occurs to various degrees. Ulceration begins much later if the tumor is planted deep in the subcutaneous tissues or in the axilla, since the incidence of surface infection is diminished. Both ulceration and peripheral expansion of the tumor continue in varying degrees until the death of the animal. Metastases have not been observed by us.

Since the ten day old tumor shows the optimal response to bacterial filtrate,[14] and since at this age it does not usually show ulceration or hemorrhage, it is taken as the model for histologic description. Differences between earlier and later stages are described relative to the ten day old tumor. The tumor cells assume one of two types of arrangement. In one, the cells grow in broad sheets without distinct cell boundaries. With the oil immersion lens, occasional cell boundaries are visible. The tumor appears to consist of a basophilic cytoplasmic mass containing large, closely packed nuclei. There are fine basophilic granules in the cytoplasm. The nuclei are large and vesicular, and each has a distinct basophilic nucleolus. The nuclear chromatin is coarse and appears in the form of large irregular granules interconnected by fine chromatin threads. In the second and more common type of arrangement, the cells are separated and show distinct cytoplasmic processes (fig. 1). Often there is a suggestion of continuity between the processes. The cells are elongated; the nuclei are smaller and darker. In edematous areas the cells assume a spindle shape. Mitoses are numerous in both forms and are usually typical. Occasional giant tumor cells are seen, especially at the periphery. A moderate number of thin-walled, endothelial-lined vascular channels are seen in the tumor; these are most often dilated and congested. Invasion of the vessels is not seen.

The ten day old tumor presents varying amounts of necrosis. It is estimated that about one third to one half of the mass is necrotic. The necrotic areas assume several forms. In one, the tumor cells are free, rounded and somewhat smaller than the original cells, with basophilic or neutrophilic cytoplasm and partially maintained nuclear structure. A second form consists of more or less indistinct, shrunken cells with eosinophilic cytoplasm and pyknotic nuclei, which, however, still retain their shape. A third form of necrosis consists of amorphous, deeply eosinophilic granular material heavily interspersed with basophilic nuclear débris. It appears that these forms represent, in order, the progressive degenerative changes of the tumor cells.

Hemorrhage occurs chiefly in small areas beneath the ulcerated skin surface and varies from small focal to large collections of erythrocytes but is never striking. Alterations of the walls of vessels are not evident.

14. Shwartzman, G.: Personal communication to the authors.

There is usually an inflammatory reaction of considerable extent around the tumor, manifested by an infiltrate of polymorphonuclear leukocytes, lymphocytes and macrophages and marked edema. Indeed, the edema is often striking. About the tumor may be seen a proliferative reaction with many young fibroblasts, which are often difficult to distinguish from isolated peripheral tumor cells. Encapsulation is not noted.

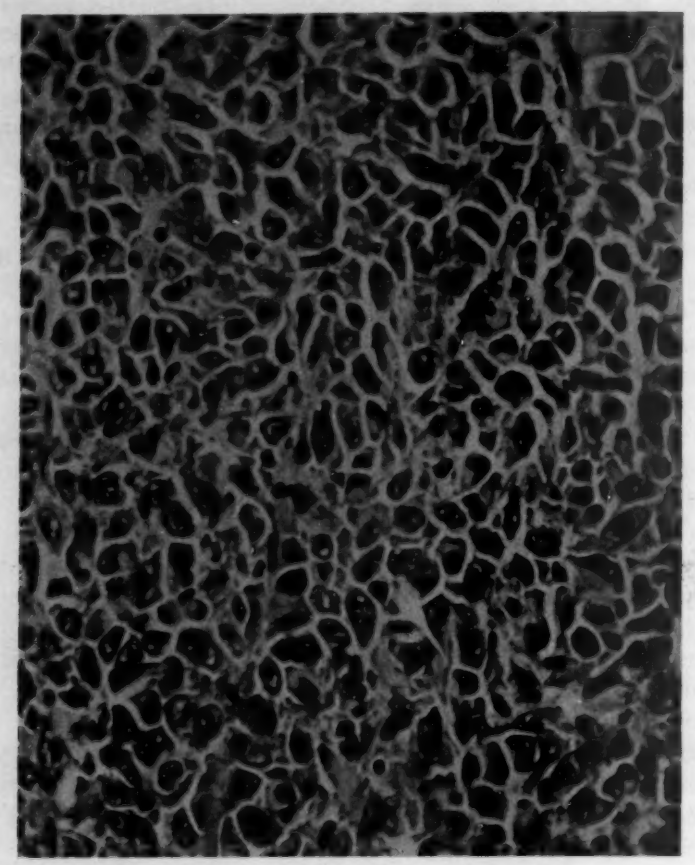

Fig. 1.—High power view of sarcoma 180 of mice, showing the characteristic appearance of the tumor.

The tumor is expansile in growth and ulcerates through the epidermis. It grows laterally by freely invading the subcutaneous fat and connective tissue, and in the depths it invades the muscle down to the peritoneum or pleura. There is only slight tendency toward destruction of tissue.

The four day old tumor consists of isolated rounded cells (some, however, with cytoplasmic processes) possessing hyperchromatic nuclei and showing only occasional mitoses. Small foci of necrotic tumor are present, undoubtedly remnants of the originally transplanted material. A moderate amount of edema and inflammatory reaction are already evident, but no fibroblastic reaction.

The tumor at the fifth and sixth days of life appears like that on the tenth day except, of course, for the smaller size. The inflammatory reaction is less marked than at ten days, and the proportion of viable to necrotic tumor is greater.

Infiltration of the epidermis may be seen already on the ninth day, and at times, also, ulceration, usually depending on the depth of the original inoculation. Tumors after the tenth day show increasingly severer necrosis and surface ulceration with a marked circumscribed inflammatory reaction. They are usually larger in size and often present small foci of hemorrhage in the ulcerated areas.

GROWTH AND MORPHOLOGIC CHARACTER OF SARCOMA 180 IN MICE TREATED WITH BACTERIAL FILTRATES

The animals were divided into two groups. One (group 2) was treated with a dose of bacterial filtrate known to produce regression of tumor growth in a high percentage of treated animals, and the other (group 3), with a dose known to cause regression in only a small percentage of treated animals.

Shwartzman [14] had found that 100 rabbit reacting units of meningococcus filtrate led to complete disappearance of about 80 per cent of tumors in animals treated at about the tenth day of tumor life. Accordingly, in our group 2 this dose of filtrate was administered to each of 104 mice bearing ten day old tumors. Seventy-six of the animals were killed from twenty-four hours to ten days later. Twenty-eight were not put to death (table 2). Of the latter, 4 presented actively growing tumors after three days and ultimately died with large ulcerating tumors. Four died between four and six weeks after treatment but at death presented no evidence of tumor. Twenty were alive thirteen or more weeks after treatment, with no evidence of tumor. Of the 28 mice not put to death, therefore, 24 showed complete regression of their tumors, or over 85 per cent, corresponding to Schwartzman's findings.

The animals of group 3 were divided into two subgroups. In one, mice bearing ten day old tumors, similar to those in group 2, were given each a single intravenous injection of from 20 to 25 rabbit reacting units of meningococcus filtrate and were then killed from four hours to nine days later. In the second subgroup mice bearing tumors from three to twelve days old were each given the same amount and killed twenty-four hours later (table 3).

Morphologic Observations on Tumors of Group 2, Receiving 100 Rabbit Reacting Units of Filtrate.—Following the intravenous injection of the filtrate, a fairly regular sequence of events was observed. Commencing at about four hours after the injection, the skin over the tumor assumed a grossly hemorrhagic appearance. This increased in intensity and became striking during the first twenty-four hours, at the end

TABLE 2.—*Group 2. Mice Bearing Ten Day Old Tumors Treated with One Hundred Rabbit Reacting Units of Meningococcus Filtrate*

Animals	Time After Injection at Which Animal Was Killed, Days
12	1
12	2
14	4
13	5
12	7
13	10*
28	Not killed
104	

* See text page 977.

TABLE 3.—*Group 3. Mice Bearing from Three to Twelve Day Old Tumors Treated with from Twenty to Twenty-Five Rabbit Reacting Units of Meningococcus Filtrate*

Animals	Age of Tumor at Time of Treatment, Days	Time After Treatment at Which Animal Was Killed, Days
3	3	1
3	4	1
3	5	1
3	8	1
15	10	1
3	10	4 hours
4	10	2
3	10	3
4	10	4
3	10	7
3	10	9
1	11	1
6	12	1
54		

of which time the surface of the tumor became dry and hard. The tumor diminished in size at this time, and by the second day a dry hemorrhagic scab was formed, with further decrease in size. At the periphery, a firm collar of tissue was still palpable. The scab gradually loosened and fell off in about seven days, leaving a fairly clean granulating surface, much smaller in size than that formerly occupied by the tumor.

Histologically, the tumors twenty-four hours after injection of the filtrate presented a rather uniform picture. The striking features were the absence of normal-appearing tumor cells, which were replaced by massive necrosis. The necrotic tumor presented early necrosis, as

described in the nontreated controls. The skin surface was ulcerated, with varying amounts of subsurface hemorrhage and moderate vascular congestion throughout. Edema was striking. Vascular alterations or thrombi were not observed.

At forty-eight hours there was progressive increase in the necrosis, and the inflammatory reaction was intensified, with increase in fibroblastic elements. In 7 of the 12 animals killed at this period, small isolated tumor cells showing discernible nuclear structure were found at the periphery of the necrotic mass (fig. 2 *A*). In 3 mice only necrotic tumor was seen, and in the remaining 2 mice characteristically normal tumor was present in focal areas, usually at the periphery, with many mitoses. As in the group killed at twenty-four hours after injection, no vascular changes were seen.

At ninety-six hours after treatment necrosis was still further advanced, with the surface ulceration more marked, and there was a striking inflammatory response, with edema. Four of the 14 mice in this group showed complete necrosis of their tumors. In 6, isolated tumor cells were found as in the forty-eight hour group, and 4 showed residual characteristic tumor, somewhat larger in extent than in the previous group.

At five days the tumors were completely degenerated in 4 of 13 animals. The small mass of degenerated tissue was surrounded by a collar of granulation tissue, which may account for the occasional impression of residual tumor on gross examination. Five other animals presented small areas in which occasional isolated tumor cells were seen, but generally fewer in number than in the forty-eight hour series. Four showed areas of actively growing tumor at the periphery. It appeared here that the tumor represented renewed growth from residual cells. In some of the growing tumors which were larger, there appeared central areas of early necrosis like that seen in controls. The regrowing tumor surrounded the necrotic tissue and appeared to push it forward toward the skin surface (fig. 2 *B*).

At seven days 7 of 12 mice showed no tumors whatsoever. In several instances there was a rather small area of ulceration with a minimal amount of residual necrotic tissue, whereas in others there was a slight surface ulceration with a base of granulation tissue. Five animals showed actively growing tumors of appreciable size, comparable to those of the five day group but larger in size.

Of the 13 mice killed ten days after injection of the filtrate, only 4 showed complete tumor regression with but a small surface ulceration covered by inspissated necrotic tissue having a granulating base (fig. 3*A*). Epithelialization was evident in several instances. The remaining 9 mice presented renewed growth of tumor surrounding

the necrotic tissue beneath the surface ulceration, and early secondary necrosis was seen in the regrown tumor. In view of the small percentage of complete regressions in this series, it was decided to reinves-

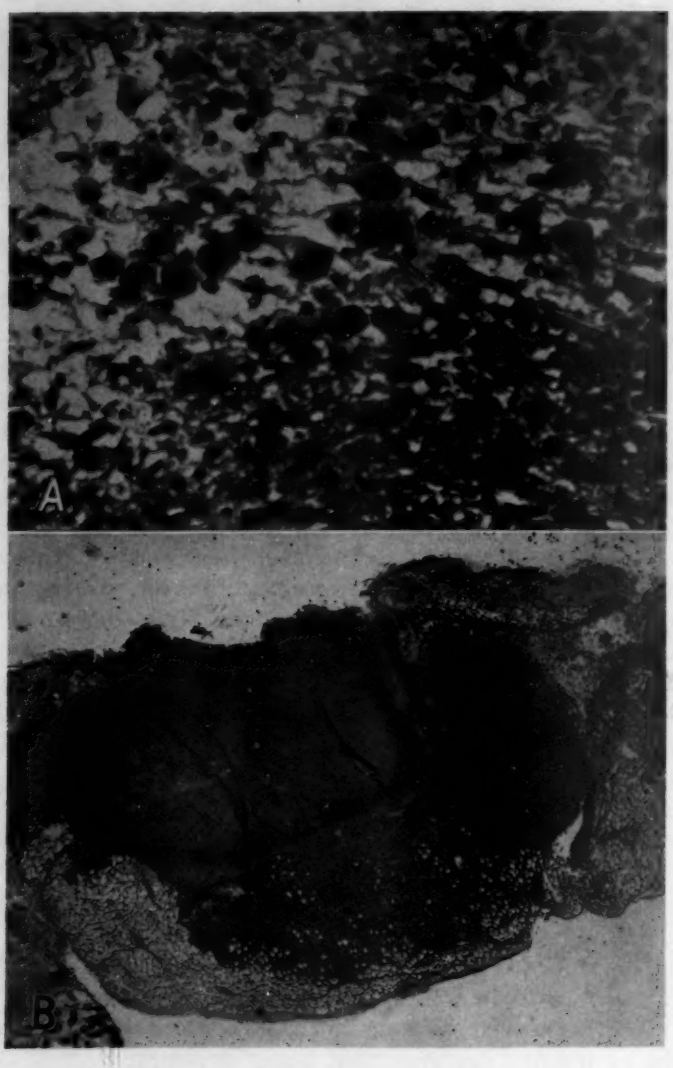

Fig. 2.—*A*, isolated tumor cells present at the periphery of a tumor. It is from such residual cells, escaping necrosis, that renewed growth of a tumor occurs. High power view, reduced. *B*, renewed growth of tumor surrounding a necrotized tumor as a collar and pushing it toward the ulcerated skin surface. Low power view, reduced.

tigate the potency of the filtrate. Retitration under the supervision of Dr. Shwartzman showed a definite loss in potency so that, instead

of 100 rabbit reacting units, the mice actually received only 75 units. A new series of mice was then treated with an active filtrate. Of 28 mice so treated, 24, or over 85 per cent, showed complete tumor regression and, as previously mentioned, 20 were alive thirteen or more weeks

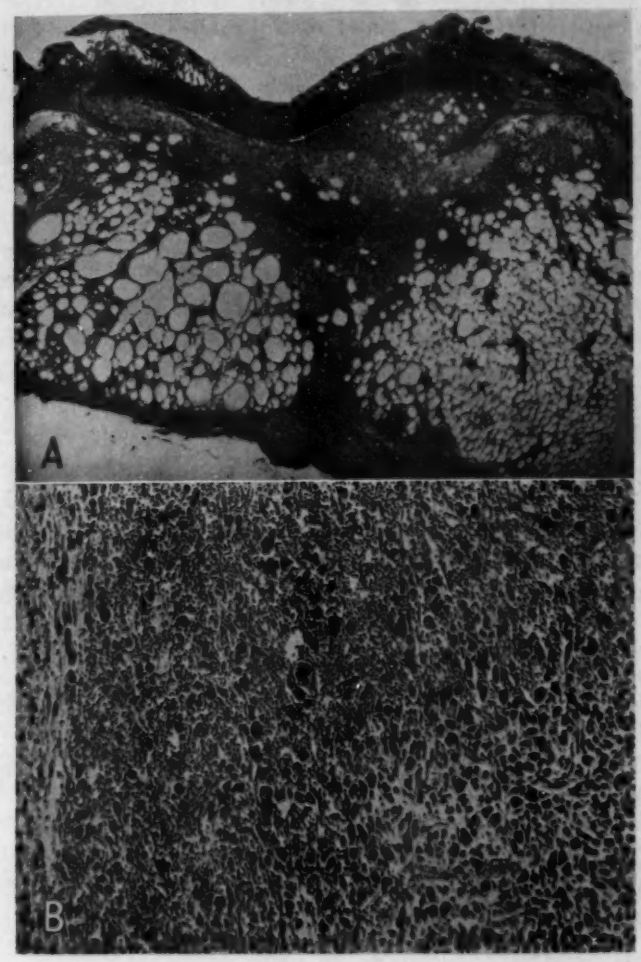

Fig. 3.—*A*, skin site of a tumor showing complete absence of tumor cells, with a granulation tissue residue and surface insipissated necrotic material. Note incipient epithelialization at the margins. Low power view, reduced. *B*, appearance of a tumor four hours after an intravenous injection of meningococcus filtrate, 25 rabbit reacting units. Note the hemorrhagic extravasation between tumor cells. Medium power, reduced.

later. It was deemed unnecessary to kill this group. In an examination of the animals it was frequently impossible to determine at what point

the tumor was inoculated, since complete epithelialization with regrowth of hair had occurred.

Despite a careful search, thrombi or other vascular alterations were not found in any of the animals in this series.

Morphologic Observations on Tumors of Group 3, Receiving from 20 to 25 Rabbit Reacting Units of Filtrate.—In the first subgroup, namely, animals bearing ten day old tumors and given from 20 to 25 rabbit reacting units of filtrate, the gross appearance of the tumors subsequent to treatment varied according to the ultimate fate of the tumors. A large number of tumors continued to grow unabated. Others went on to complete regression and differed in no way, grossly or microscopically, from those in group 2. A number, however, showed apparent retardation of growth for several days only to show subsequently progressive growth. Those tumors which did not regress showed histologically a smaller amount of residual tumor, however, than did untreated controls of the same age period. This was true only up to seven days after treatment. Subsequently, there was no difference between inadequately treated animals and untreated controls.

In the second subgroup it was found that three, four and five day old tumors had not responded to the injection of filtrate when examined twenty-four hours after treatment. This finding corresponds to the macroscopic observations noted by Duran-Reynals [4] and others. The response of eight, eleven and twelve day old tumors was similar to that of ten day old tumor when a like amount of filtrate was administered.

COMPARATIVE STUDIES

It has been demonstrated fairly conclusively that irradiation effects destruction of animal tumors by a combination of direct action on the tumor cells and indirect action on the stroma and surrounding tissues. Sugiura [15] found this to be true for sarcoma 180 of mice irradiated in vivo. We treated a group of 9 mice bearing ten day old sarcoma 180 with a single dose of 2,500 roentgens. The animals succumbed from one to eight days later. Microscopic studies revealed a more pronounced inflammatory reaction than in those treated by injection of meningococcus filtrate, and in addition bizarre tumor cell forms were present, as well as many giant cells in residual islands of tumor. This picture was not seen in filtrate-treated animals.

Duran-Reynals,[4] in an extensive study of the effect of bacterial filtrates on spontaneous and transplantable animal tumors, found that slowly growing malignant spontaneous or transplantable tumors were resistant to the effects of intravenous injections of Bacillus coli filtrate.

15. Sugiura, K.: Radiology **28**:162, 1937.

This was also true of rapidly growing malignant tumors which were transplanted into heterologous hosts. Carcinoma 11 and 63 of mice, a tumor which is known to be slow growing although malignant, was transplanted into a number of mice, of which 24 were treated with 100 rabbit reacting units of meningococcus filtrate. The filtrate was without effect on the tumor. In a selected strain of mice we obtained takes in only 30 per cent.

Fogg [8] treated mice bearing dibenzanthracene tumors with bacterial filtrate. He obtained complete healing in 4 of a group of 42 animals. We gave 10 mice bearing seventh generation transplants of a dibenzanthracene tumor 100 rabbit reacting units of meningococcus filtrate intravenously and killed the mice from twenty-four to seventy-two hours later. Hemorrhage and necrosis were observed in the tumors in varying degrees. Some showed almost complete necrosis, with but a small periphery of residual tumor; others showed only a small central area of necrosis with a wide zone of surviving tumor.

COMMENT

The microscopic studies of three, four and five day old tumors did not throw any light on the question of their lack of response to intravenously administered bacterial filtrate. At the eighth and ninth days of tumor life the reactions were comparable to that seen at ten days, and because of the optimal size of the tumor at ten days, together with the absence of secondary changes, the tumor of this age was taken as the model for experimental studies.

As previously described by Shwartzman,[5b] the tumor presents marked hemorrhage four hours after the intravenous injection of bacterial filtrate (fig 3 *B*). The extent of the hemorrhage varies with the dose employed. After the hemorrhage, the tumor surface becomes dry and hard, and there is noticeable shrinkage of the tumor. This is so striking that by the third day after treatment the tumor is reduced to about one-fourth the size of controls of the same age period.

A similar series of changes is seen in tumors which do not go on to complete regression. However, at some time between the fifth and tenth days after treatment renewed growth occurs from the peripherally situated residual tumor cells, and the subsequent course is the same as in untreated control tumor mice.

Histologically, the outstanding features seen initially are hemorrhage, vascular engorgement and edema. These are followed rapidly by extensive necrosis of tumor cells. The hemorrhagic response is seen chiefly within four hours after the intravenous injection of the filtrate and tends to diminish at twenty-four hours. At later periods there is little evidence of hemorrhage. It does not appear that hemorrhage alone is responsible

for the extensive necrosis of tumor cells which ensues. Although the hemorrhage is diffuse, involving almost the entire tumor, it is neither massive nor apparently severe enough to cause the widespread necrosis which is seen within twenty-four hours after injection of the filtrate. That other factors are involved in the destruction of the tumor is evidenced by the fact that at later periods, namely three days after treatment or later, well maintained tumor cells are frequently still present, and hemorrhage is already absent, yet ultimately such tumors may regress completely. The source of the hemorrhage is still in doubt, since capillary rupture is not evident.

Although constantly sought for, vascular alterations were never encountered. Thrombosis was not seen in any instance.

The degeneration of the tumor cells following intravenous injection of the filtrate is extensive within twenty-four hours, and its extent appears to be a function of the quantity of filtrate injected. Fogg pointed out that if tumor cells persist at the base after treatment, renewed growth of tumor will usually occur. Histologically, animals treated with as much as 100 rabbit reacting units of filtrate often showed residual isolated cells or nests of cells at the periphery. Later stages revealed actively growing tumor in such peripheral areas. Although the renewed tumor growth very early in its course showed spontaneous secondary degenerative changes, further proliferation nevertheless continued until the animal died.

The inflammatory reaction seen about the tumor in controls appears to be intensified in treated animals, yet on the whole it does not seem to be of sufficient intensity to account for the death of the tumor.

Examination of the internal organs in about one half of the animals revealed neither metastases nor other significant changes.

The dramatic response of the tumor with hemorrhage and necrosis, plus the intensification of an already existent inflammation, is comparable to the changes seen in the phenomenon of local tissue reactivity (Shwartzman phenomenon). The characteristic thrombosis seen in the local tissue phenomenon, however, is lacking here.

The effect of the filtrate on the tumor appears to be essentially one of direct action on the cells, which is in agreement with the findings of Apitz, Andervont and others. The quantitative character of the reaction is evidenced by the higher percentage of complete regressions when larger doses of filtrate are employed.

SUMMARY

When a mouse bearing sarcoma 180 is given an intravenous injection of meningococcus filtrate a definite series of changes occur within the tumor. There is a preliminary brief period of congestion and hem-

orrhage, which reaches its maximum approximately four hours after the injection. This is followed by necrosis of the tumor cells and extensive edema. If the dose is sufficiently large (about 100 rabbit reacting units) the entire tumor subsequently sloughs out, and healing follows with a granulation tissue response and restitution of the surface epithelium. With a smaller dose, renewed growth of the tumor takes place from residual undestroyed tumor cells, usually located at the periphery. Thrombi or other vascular alterations are not seen. In agreement with the findings of other investigators, the effect of the intravenously injected filtrate on the tumor appears to be directly on the tumor cells.

EFFECT OF SYMPATHECTOMY ON THE VASA VASORUM OF THE RAT

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AND

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It is generally agreed that, following sympathectomy, changes in the peripheral vessels that possessed a sympathetic innervation are as follows: 1. An immediate dilatation appears and remains marked for somewhat less than a week. 2. At the end of that period the dilatation becomes less but is still somewhat above normal. 3. Subsequently the denervated area fails to respond to stimulation of the sympathetic nervous system. The first effect, being the most marked, is the easiest to detect experimentally. Changes in cutaneous temperature are the most reliable guide. This procedure is not available, however, for a study of the deeper structures. These may be investigated by injecting some form of particulate matter into the vessels, which circulates through them and may be detected in them in histologic sections. While it may be assumed that the vasa vasorum would share in the general response of blood vessels to sympathectomy, this has never been demonstrated so far as we know.

Investigators have injected particulate matter into the general circulation and have subsequently studied sections of symmetric parts or organs. If equal amounts of particulate matter were found in both sections, some have assumed that the rate of circulation in the one organ or part was the same as in the other. This is not necessarily a proper inference. The correct inference may be only that in the one there was the same number of vessels open as in the other. If time is given for even distribution of the particulate matter throughout the entire circulation, the particles will presumably be found in the lumens of all vessels through which blood is passing and which are capable of admitting the particles. Yet the rate of flow may be quite different in different vessels. Only if the examination is made soon enough after the injection will the particulate matter have failed to reach areas with

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relatively sluggish circulation although already demonstrable in areas with more rapid circulation.

When particulate matter has been injected, conclusions may be drawn from sections made subsequently, as follows: 1. Circulation was greater in a part or organ where more of the injected material is found

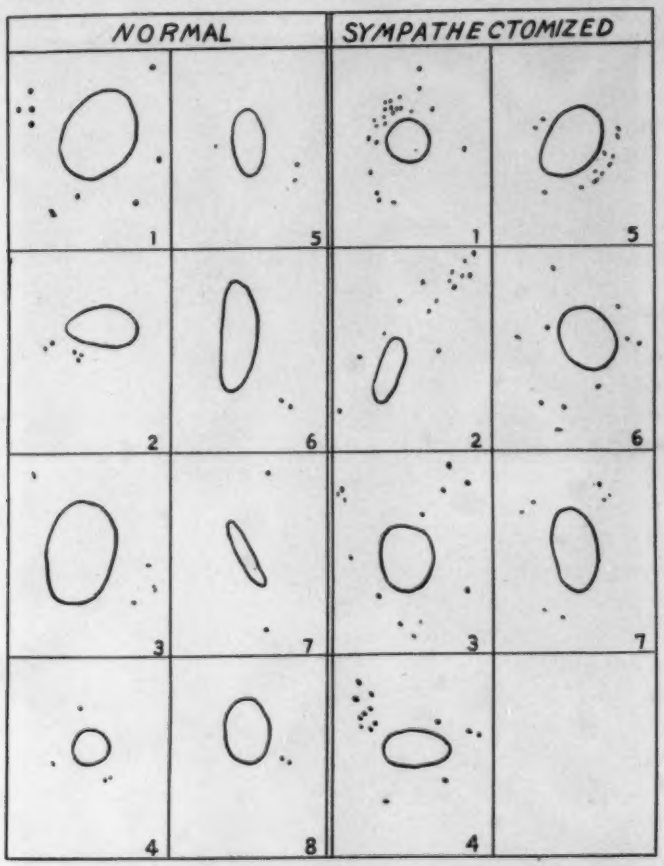

Fig. 1.—Tracings of camera lucida drawings of the areas about the femoral arteries in all the animals. The lumens of the vessels and the carbon particles are shown. Normal animals are represented by drawings *1* to *8* on the left and animals having a previous lumbar sympathectomy by drawings *1* to *7* on the right. For a description of the areas see the text.

than in a corresponding part or organ showing less, either because more vessels are open or because flow was more rapid and the vessels more quickly filled. 2. If the amounts of injected material in two corresponding areas are identical, the numbers of open vessels are also identical, and either the rate of circulation was the same in both areas

or the interval of time was sufficiently long to allow complete replacement of the circulating fluid in the part or organ with the slower circulation.

METHOD

Carbon particles are readily identified in unstained sections. Hydrokollag 300 [1] prepared according to the method of Drinker [2] (who used it for a somewhat

Fig. 2.—Untouched photographs of the areas observed in normal rat 4 (upper) and sympathectomized rat 5 (lower). The sections are unstained, and the photographs were taken with the light considerably reduced.

different purpose) is well tolerated by the animal, but the particles are extremely small and difficult to identify. Higgin's black ink diluted with an equal amount of

1. This substance was obtained from Pfaltz and Bauer, Inc., 300 Pearl St., New York.

2. Drinker, C. K., and Churchill, E. D.: Proc. Roy. Soc., London, s.B **101**: 462, 1927.

distilled water is tolerated nearly as well, and the particles are more easily recognized. After we had made about fifty experiments with hydrokollag 300, we changed to ink. To compare injections in different animals, one must use ink from the same reservoir and inject it in the same working period.

Unilateral lumbar sympathectomy is, except in an occasional animal, impossible in the rat because of the proximity and communications between the two lumbar chains. Therefore 8 normal rats were compared with 7 on which bilateral lumbar sympathectomy had been performed five days before the injection. Each rat was wrapped in a towel with the right leg exposed, and under local anesthesia, obtained with procaine hydrochloride, 1 cc. of the ink mixture was injected into a superficial leg vein in about thirty seconds. The amount given was the same in all animals, as the variation in weight for the entire group did not exceed 10 Gm. The animal was released from the towel and permitted to move freely about the cage. There was no outcry or other evidence of excitement. Five minutes after the injection the rat was killed by a blow on the head, and within five seconds the left leg was severed from the body. The tissue about the femoral vessel, midway between the hip and the knee joint, was fixed in solution of formaldehyde U. S. P., and frozen sections were cut 100 microns thick. These were mounted in balsam and examined unstained. For orientation, alternate sections were stained with hematoxylin and eosin or simply with eosin. The carbon injected was studied only in the unstained sections. The area that included the femoral artery was examined microscopically (16 cm. objective) with the light cut down. Then the light was increased, making the tissues partially invisible while the carbon particles stood out clearly. All the sections from the normal rats were repeatedly compared with all the sections from the sympathectomized animals.

RESULTS

The visible carbon in the walls and periadventitial spaces of the arteries of each of the sympathectomized rats was greater in amount than in 7 of the 8 normal controls. This is shown graphically in figure 1 *A*, which is a tracing of camera lucida drawings of the femoral arteries of all the animals. The lumen of each artery and the carbon particles are shown. No attempt was made to represent the size and shape of each carbon particle. The series is graded roughly in descending order; i. e., in the normal group rat 1 has a heavier injection than rat 2, and so on. It is obvious that rats 1 to 7 in the sympathectomized group have heavier injections than rats 2 to 8 in the normal group, but that rat 1 in the normal group has as heavy an injection as rats 6 and 7 in the sympathectomized group. Figure 2 shows photographs of normal rat 4 and sympathectomized rat 5. For orientation, the photograph was taken with the light considerably cut down so that the carbon does not stand out as clearly as it otherwise would.

CONCLUSION

The number of open vasa vasorum in the femoral artery of the rat is increased five days after lumbar sympathectomy.

EXPERIMENTAL GASTRIC ULCER (PITRESSIN EPISODES)

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The literature dealing with clinical, experimental and postmortem observations on the pathogenesis of gastroduodenal ulcers presents many conflicting views. Nevertheless, if direct traumatic agents (chemical, physical, etc.) are excluded, all the findings point to the same fundamental cause of ulcer formation, namely, local nutritional inefficiency, transient or prolonged.

Practically all the many ingenious ways of producing peptic ulcers experimentally involve the integrity of the local blood supply and lead in one way or another to nutritional changes in the walls of the stomach or duodenum. The selective location of ulcers in the lesser curvature and in the duodenal bulb is easier to understand since Reeves [1] reemphasized the fact that the anatomic particularities of the lesser curvature of the stomach and of the duodenal bulb are quite different from those of other parts. He found that the circulation in the vessels of the submucous arterial plexus of the lesser curvature tends to be slow by comparison with that in other parts of the stomach. The vessels are much smaller, their length is greater, and there are fewer anastomoses. He also found that in the submucous plexus of arteries in the duodenal bulb the vessels are smaller, are fewer and do not anastomose so freely as those in other parts of the duodenum. Such anatomic peculiarities under certain conditions will lead to nutritional changes in this region that predispose to ulcer formation. In connection with these anatomic differences studies have been made dealing with alterations of the ganglions and nerve tissues of the mucosa and submucosa which may have direct bearing on functional disturbances of the vessels.

The various intracranial lesions that have been produced experimentally, with stimulation of the vagus or paralysis of sympathetic nerves, may also lead to formation of peptic ulcers in regions that are more abundantly supplied with nerves, whose action is reflected in vascular dysfunction.

Ever since the time of Virchow, pathologic changes in the blood vessels have been observed leading to vascular obstruction or stasis and

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1. Reeves, T. B., in Collected Papers of the Mayo Clinic, Philadelphia, W. B. Saunders Company, 1918, vol. 10, p. 3.

ultimately through impairment of the nutrition to ulcer formation. Konjetzny [2] related ulcer formation to preexistent and coexistent duodenitis and gastritis, conditions which are also intimately connected with vascular activity.

Clinically, it is recognized that persons with peptic ulcers are usually of an asthenic habitus, with vasomotor hyperirritability. The "undoubted influence" (Stewart [3]) of sudden changes of temperature in the production of relapses of gastric and especially of duodenal ulcers and the frequent association of a poor peripheral circulation with gastric and duodenal ulcers have also been noted.

Trauma from blows on the abdomen with later development of peptic ulcers has been observed, thus involving the reflex action from the surface to the stomach structure. Lately, some authors (S. C. Robinson [4]) have spoken of the role of emotions in the formation of gastroduodenal ulcers, pointing to the fact that emotional upsets may result in visceral dysfunction, with an outcome in ulcer formation. Von Bergmann,[5] who expressed the belief that constitutional disharmony in the vegetative nervous system is the essential factor in the development of all ulcers, pointed to the compression of the blood vessels in the associated spasm of the muscularis mucosae and muscular layers of the stomach.

All this definitely places emphasis on the behavior and the state of the blood vessels as the main and intimate cause of ulcer formation. With normal function and normal nutrition of the gastroduodenal walls, ulceration is prevented. Any interference with their function or disarrangement of function may lead to pathologic changes in the corresponding regions which ultimately will lead to ulcer formation.

EXPERIMENTS

Pitressin (betahypophamine) causes contraction of small arterioles and capillaries. Its action is not uniform in organs or systems of organs in different animals or in different seasons. This activity of injected pitressin involves a spasm of the small blood vessels. If pitressin is injected into animals once or repeatedly, thus causing an anoxemic state in the tissues, objective evidence of alteration of the tissues can be obtained. This has been shown in producing experimental

2. Konjetzny, G. E., in Henke, F., and Lubarsch, O.: Handbuch der speziellen pathologischen Anatomie und Histologie, Berlin, Julius Springer, 1929, vol. 5, p. 768.

3. Hurst, A. F., and Stewart, M. J.: Gastric and Duodenal Ulcer, New York, Oxford University Press, 1929.

4. Robinson, S. C.: Illinois M. J. **71**:338, 1932.

5. von Bergmann, G., in Mohr, L., and Staehelin, R.: Handbuch der inneren Medizin, ed. 2, Berlin, Julius Springer, 1926, vol. 3, p. 703.

bacterial and bland endocarditis (Nedzel [6]) and in studies of bacterial localization in the central nervous system (Nedzel [7]). It has been shown previously by Dodds and his co-workers [8] and by Nedzel [9] that in animals pathologic lesions may be produced in the gastrointestinal tract by injecting extracts of the posterior lobe of the pituitary gland. These lesions include multiple hemorrhages, erosions and small and large ulcers of the stomach and duodenum, resembling those seen in man.

This paper presents a detailed study of ulcer formation due to pressor episodes evoked by injections of pitressin. All the experiments were made on dogs (usually young) by administering a single injection or repeated injections of pitressin intravenously. The dose has been 20 pressor units per five kilograms of weight of the animal. The repeated injections were given at the rate on an average of two injections per week up to fifty injections. The total number of dogs used was 62, of which 39 (63 per cent) gave negative and 23 (37 per cent) positive results. The latter animals showed lesions of the stomach and duodenum ranging from a small erosion up to a large ulcer. A few of the dogs died from causes not related to gastric lesions; the majority were put to death.

DIRECT OBSERVATIONS ON THE MUCOSA

Before describing in detail the histologic observations made during the course of the development of gastric ulcers under such conditions, I shall first delineate the observations made by direct inspection of the mucous membrane of the living dog when subjected to intravenous injection of pitressin under anesthesia obtained with phenobarbital sodium.

Through an incision about 15 cm. long in the upper part of the abdominal wall, in the median line, the stomach was exposed. The latter was opened by an incision in its front wall, near the pylorus, and the gastric mucous membrane in this region made visible. The contents of the stomach, if any, were gently removed, and hemorrhage from the incision was stopped. The exterior of the stomach was constantly bathed with warm saline solution (at the temperature of the animal) and the injection of pitressin was made (femoral vein). Attempts at direct photography failed because of the movements of the stomach after the injection of pitressin and because of the respiratory movements of the diaphragm.

The degree of reaction differed in different dogs, but all reacted similarly. In a minute or more after the injection of pitressin the

6. Nedzel, A. J.: Arch. Path. **24**:143, 1937.

7. Nedzel, A. J.: Proc. Soc. Exper. Biol. & Med. **34**:239, 1936.

8. Dodds, E. C.; Noble, R. L., and Smith, E. R.: Lancet **2**:918, 1934. Dodds, E. C.; Noble, R. L.; Hills, S. M., and Williams, P. C.: ibid. **1**:1099, 1935.

9. Nedzel, A. J.: Proc. Soc. Exper. Biol. & Med. **34**:130, 1936.

Fig. 1.—Mucous membrane of a dog's stomach, somewhat swollen and reddened, with multiple hemorrhages and small erosions; × 1. The dog (no. 1), between June 5 and June 11, 1934, when it was killed, received three injections of pitressin.

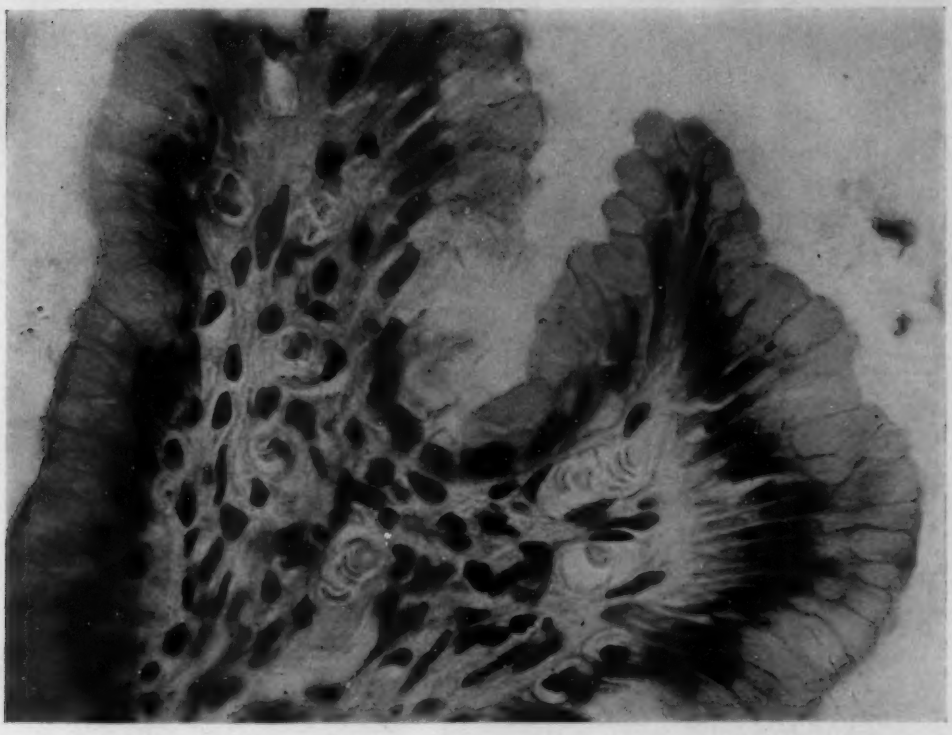

Fig. 2.—Surface of a dog's gastric mucous membrane twenty-five minutes after an injection of pitressin; × 808.6; oil immersion. The capillaries are widely dilated and packed with erythrocytes.

stomach contracted, and the folds of the mucosa became more pronounced and paler. A few minutes later the stomach dilated, the height of the folds was still increased, and red dots appeared on the surface without visible rupture of the mucosa. These dots enlarged to from 1 to 3 mm. in diameter. On careful swabbing with cotton pledgets they disappeared transiently only to reappear in the same or adjacent places, definitely becoming visible hemorrhages. In about from thirty to forty-five minutes, especially after repeated injections (two or three injections) of pitressin, these small hemorrhages defi-

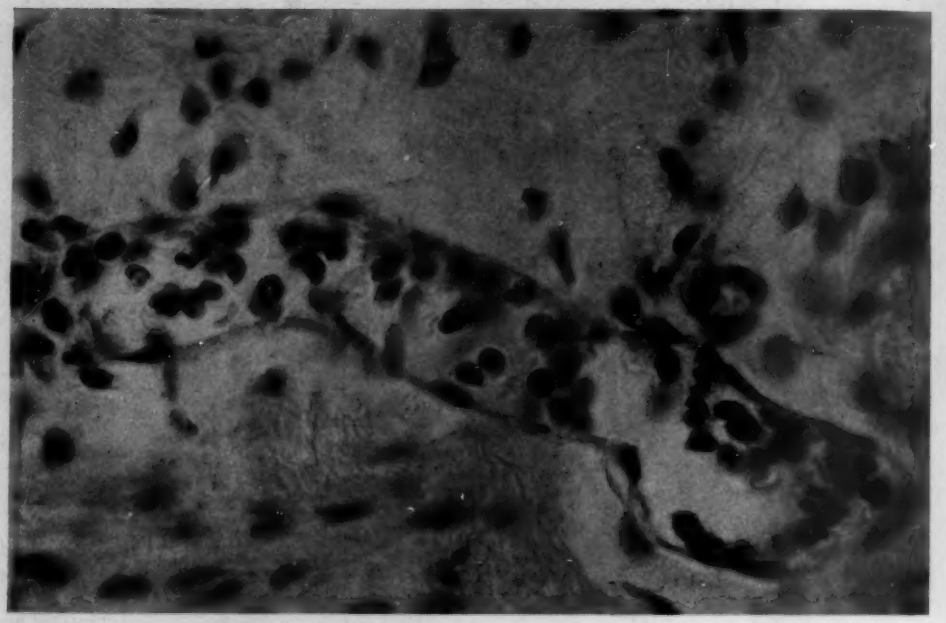

Fig. 3.—Mucosa of a dog's stomach twenty-five minutes after an injection of pitressin, showing a venule widely dilated and filled with polymorphonuclear leukocytes; × 870; oil immersion. At the right, above the vessel is a small capillary with swollen endothelium.

nitely began to resemble small erosions, and the mucosa became hyperemic with the folds disappearing (fig. 1).

HISTOLOGIC OBSERVATIONS

Early Changes.—At different stages of the observed changes, specimens of tissue were taken from the stomach and studied histologically.

Figure 2 presents an upper part of a gastric mucous membrane twenty-five minutes after an injection of pitressin. The capillaries

beneath the epithelial layer are highly dilated and filled with the erythrocytes.

Figure 3 shows a specimen taken from the same animal after the same interval of time. The small vein is filled with leukocytes, mostly polymorphonuclears. At the right, above the vein, is a small capillary with swollen endothelium. This is in the muscular layer.

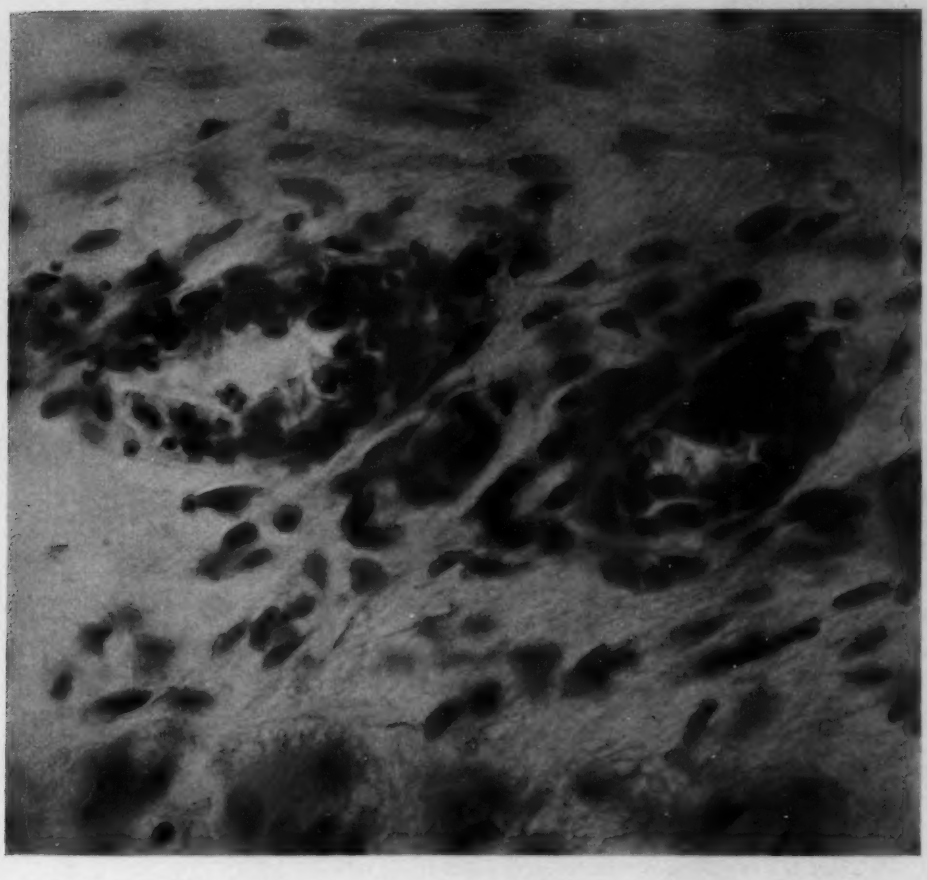

Fig. 4.—Muscular layer in the same slide as the mucosa shown in figure 3; × 870; oil immersion. The venule at the left is filled with polymorphonuclear leukocytes. At the right is an arteriole, thrombotic, the center filled with fibrin and red blood corpuscles, while to the margin leukocytes are attached, some perivascular.

Figure 4 is from the same slide as figure 3, and the tissue shown is also from the muscular layer. To the left is a vein filled with leukocytes, accumulated at its wall, mostly polymorphonuclears, and to the right is an arteriole plugged in the center with a thrombus formed of

fibrin and red blood cells, while at the periphery are leukocytes, some of which are situated perivascularly.

Figure 5 presents an upper part of the mucous membrane of a specimen taken thirty minutes after an injection of pitressin. On its surface is an exudate. The epithelial cells are filled with some débris. The capillaries are highly dilated. The interstitial tissue is somewhat edematous. Round cells are scattered. The lymph channels are dilated.

Fig. 5—Upper part of gastric mucous membrane thirty minutes after an injection of pitressin; × 256. A surface exudate is seen. The capillaries, both blood and lymph, are widely dilated. The interstitial tissue is somewhat edematous. There is a beginning accumulation of small round cells.

Figure 6 presents a small hemorrhage in a dog's gastric mucosa, in a specimen taken thirty minutes after an injection of pitressin. The blood vessels in the submucosa are widely dilated. One of them has been so overfilled that it has ruptured through the muscularis submucosa and the mucosa to the surface, destroying the muscularis submucosa in small part and the mucous layer in larger part. The epithelial layer of mucosa has also been destroyed, permitting the escape of the blood on the surface of the mucous membrane. To the left of the hemorrhage

is seen a widely dilated capillary of the mucosa. The surface of the mucosa is covered with fibrin.

Figure 7 shows a hemorrhage in the mucosa of a dog's stomach, from which the specimen was taken thirty-five minutes after an injection of pitressin. The whole mucous layer in this stomach appeared shrunken. No blood vessels are seen, except the mentioned accumulation of blood in a small hemorrhagic zone.

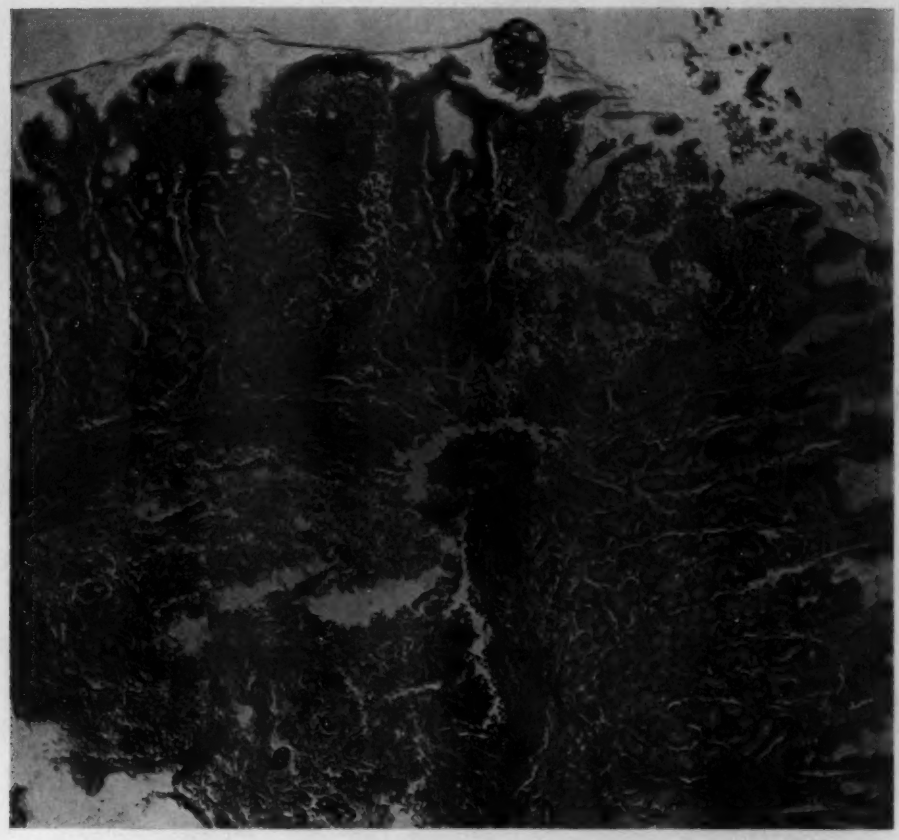

Fig. 6.—Hemorrhage; × 20. The submucosal vessels are engorged; one has ruptured with resulting hemorrhage into the muscularis submucosa, penetrating through to the mucosal surface, with destruction of tissue. Blood cells have escaped to the surface of the structure.

Later Changes.—These are the initial stages of development of the gastric ulcer due to injections of pitressin as observed in living animals. The findings presented now are from animals put to death at varying intervals of time after pressor episodes induced by injections of pitressin.

Figure 8 shows a late effect of the action of pitressin in dog 2. This animal received intravenous injections of pitressin Feb. 24, Feb.

25 and March 3, 1937. It was killed March 9. Widely dilated blood vessels are observed in the mucous membrane of the upper and middle portions of the specimen. Distended blood vessels are also found between the muscularis submucosa and the mucous membrane. The nuclei of the cells of the glands and interstitial tissue are opaque, and the whole mucous membrane is somewhat edematous. The epithelial layer is comparatively well preserved.

Fig. 7.—Submucosal hemorrhage thirty-five minutes after an injection of pitressin; × 110. The mucosa appears shrunken, and the vessels are contracted, only the hemorrhagic region bring objective evidence of the disturbance.

Figure 9 shows a part of the same slide. The blood vessels of the submucosa are highly distended. The interstitial tissue is edematous. The blood vessel in the center is filled with leukocytes, mostly polymorphonuclears.

Figure 10 shows early necrosis in a specimen of mucous membrane from dog 3, which received an injection of pitressin Jan. 1, 1934, and was killed in twenty-four hours. One sees only shadows of the nuclei, if any, of the glandular cells, as well as of those of the interstitial tissue. Below this faintly stained area is a row of scattered mononuclears.

Figure 11 presents a specimen of mucous membrane from the same dog but from another part of the stomach. There is a focus of disintegration in the central part of the mucous membrane, resembling a faint isle in the comparatively normal-appearing mucous membrane.

Figure 12 shows a late lesion in dog 4, which received injections of pitressin Feb. 11, 18, 22 and 24, 1937, and was put to death March 2.

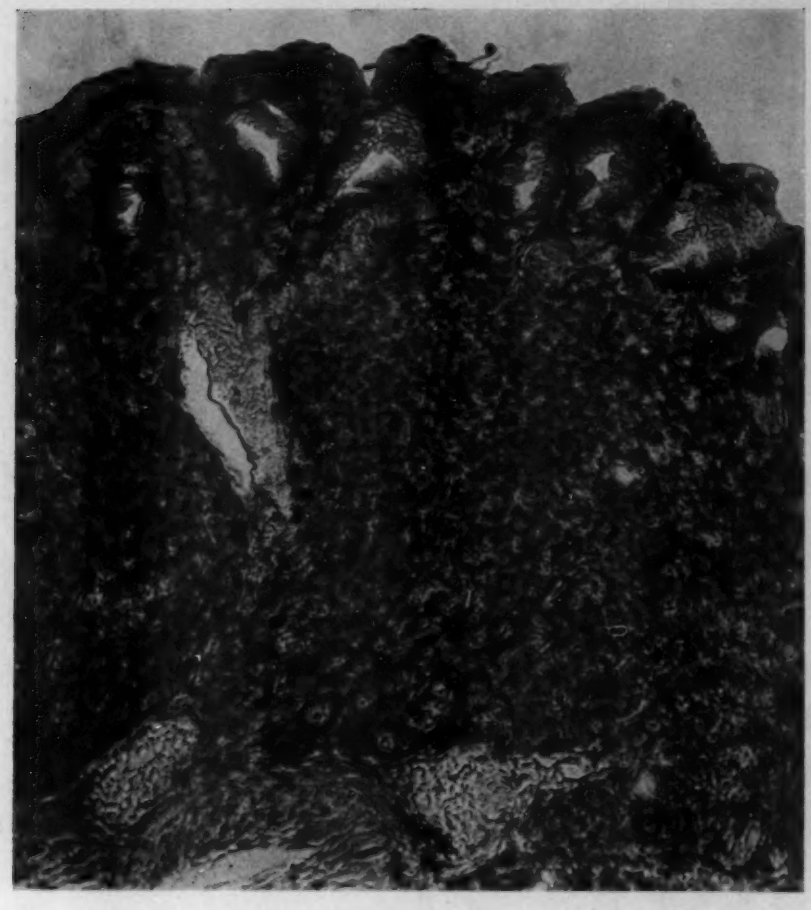

Fig. 8.—Late pitressin effect; × 110. The specimen is from dog 2, which received intravenous injections of pitressin Feb. 24, Feb. 25 and March 3 and was put to death March 9, 1937. Note the engorged vessels of the upper and middle portions of the mucous membrane. The tissues are edematous. The nuclear outlines in glandular structures and interstitial tissue are diffuse.

Macroscopically the mucous membrane appeared swollen and reddened. In the photomicrograph the epithelial layer appears unchanged. On its surface there is a layer of fibrinous exudate with formed elements,

which has permeated through the epithelial layer. In the center, beneath the epithelium, is seen a conglomeration of formed elements, mostly mononuclears. The mucous membrane is edematous in toto but mostly in its interstitial tissue. The nuclei of the glandular cells are deeply stained.

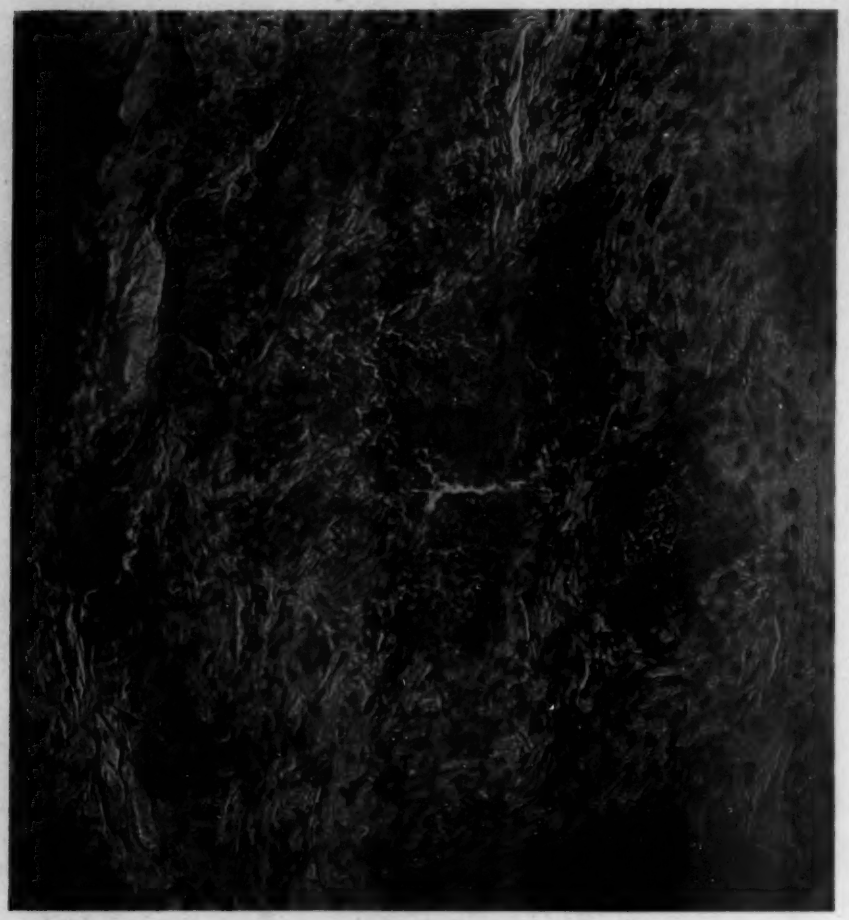

Fig. 9.—Late pitressin effect in the same slide as that shown in figure 8; × 180. Note vascular engorgement, edema of the interstitial tissue and accumulation of polymorphonuclear leukocytes in the vascular beds.

Figure 13 is from the same specimen. The whole mucosal and submucosal structure is edematous, showing cystlike accumulations of the serous exudate. The surface epithelium is absent, and the glandular tissue beneath the surface has partly disintegrated. The surface is covered with fibrinous exudate, most of it invaded by connective tissue

cells with formed elements. The superficial erosion is also filled with the fibrinous exudate containing formed elements. All over the mucous membrane are seen a number of scattered mononuclears.

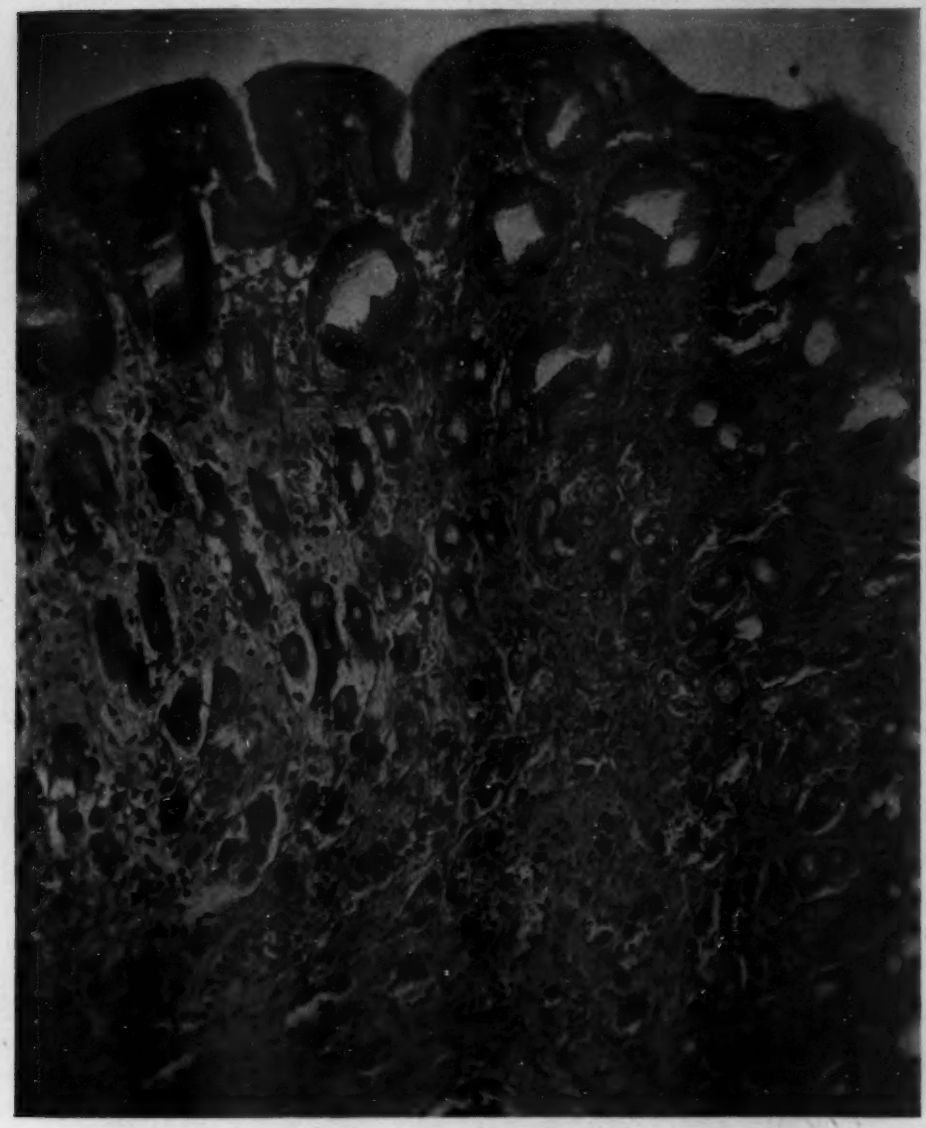

Fig. 10.—Early necrosis; × 110. The specimen is from dog 3, which received an injection of pitressin Jan. 1, 1934, and was killed in twenty-four hours. Note necrosis of the mucous membrane and edema of the adjacent margin.

Figure 14 is from the same specimen. To other characteristic features described in figures 11 and 12, here is added an erosion, which

has been filled with the exudate and also shows proliferation of the fibrocytes through this exudate (healing?).

Figure 15 shows a superficial erosion in the stomach of dog 5. Between March 3 and July 17, 1937, this dog received twenty-one injec-

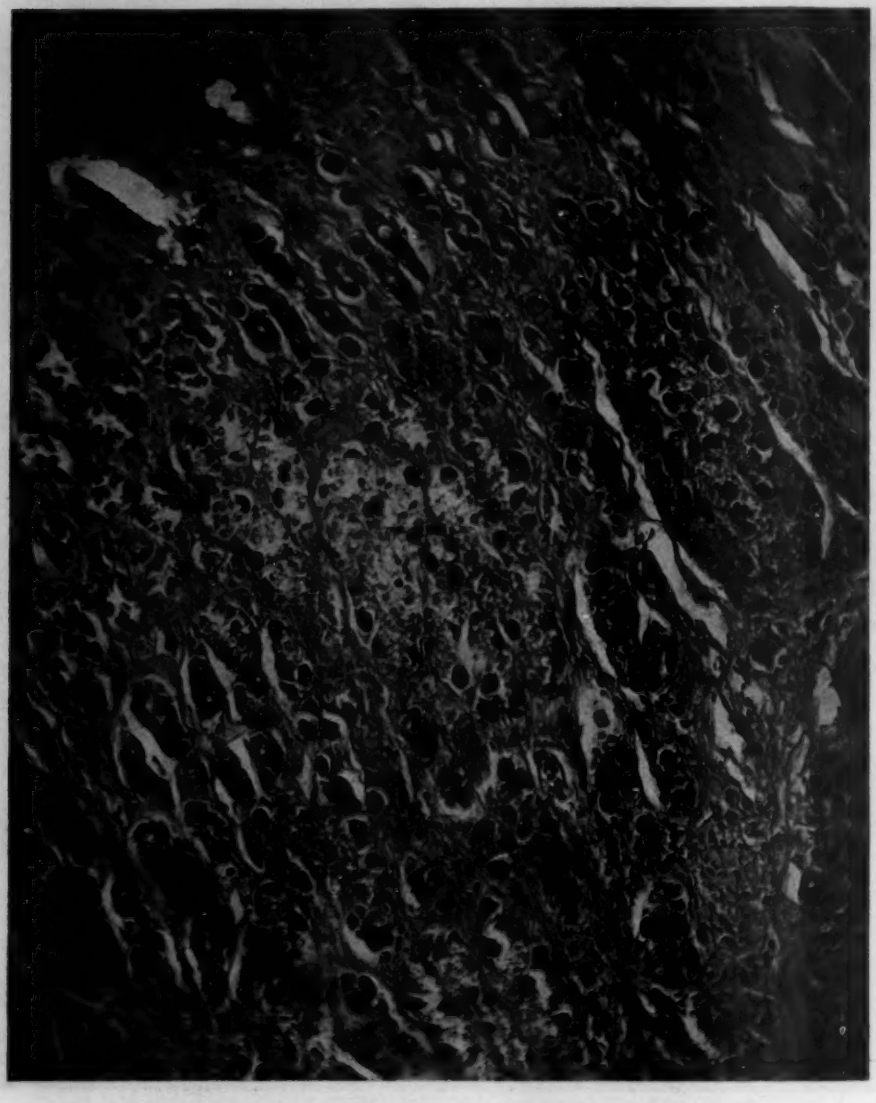

Fig. 11.—Early necrosis; × 110. The specimen is from the same dog as that in figure 10. Note focal necrosis, with edema of interstitial tissue.

tions of pitressin. It was put to death July 21. The stomach contained a number of erosions and two small ulcers, one of which is present

here. The ulcer extends from the surface of the mucous membrane to the muscularis submucosa. There is some fibrinous exudate with moderate numbers of formed elements. The edges of the ulcer are edematous, with necrosis of the structures of the mucous membrane.

Fig. 12.—Late lesion; × 110. The specimen is from dog 4, which received injections of pitressin Feb. 11, 18, 22 and 24 and was killed on March 2, 1937. Note the organizing surface exudate. The submucosa is edematous, and there is an occasional collection of proliferating interstitial cells.

Figure 16 shows an ulcer from dog 6. This animal received between April 6 and July 25, 1934, thirty-five injections of pitressin and was put to death July 25. In the stomach was found this small single ulcer. It extends from the surface into the submucosa, perforating the muscu-

laris submucosa. In the submucosa is seen a serous exudate. The edges of the ulcer show necrosis of the wall structures. The muscular layer is edematous.

Figure 17 presents an ulcer from dog 7. Between Aug. 3 and Aug. 10, 1937, when the dog was put to death, it received twelve injections at the rate of two a day. The stomach showed several small ulcers. The one presented here penetrates from the surface deep into the submucous layer, its fundus formed by the muscular layer. To the

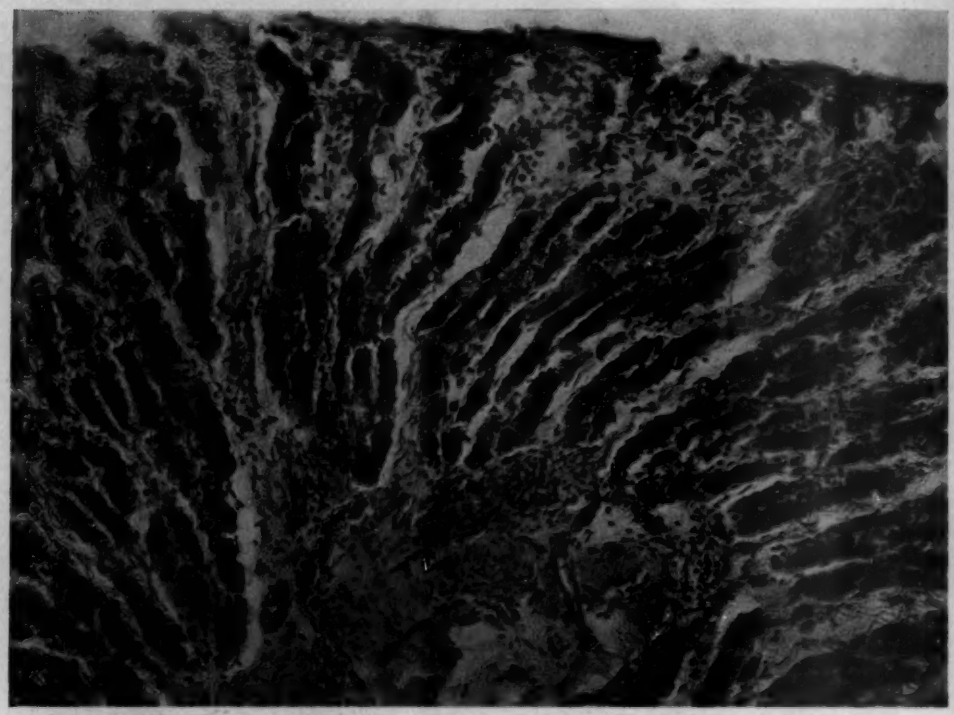

Fig. 13.—Surface scar; × 846. This is from the same dog as the lesion in figure 12. Note edema of the mucosa and submucosa with a cystlike accumulation of exudate, partial ablation of the mucosal surface with disintegration of underlying glandular tissues, the surface covered with fibrinous exudate, in part organizing, and the superficial erosion, in part filled with fibrinous exudate containing formed elements. Mononuclear cells are seen throughout the tissue.

right is seen also a partial involvement of the latter. On the left, in the submucosal layer, just above the muscular layer is a blood vessel with a thrombus completely obstructing its opening. The ulcer contains some fibrinous exudate and débris of the tissue of the mucous membrane and submucosa.

Figure 18 shows an ulcer in the stomach of a dog 8. Between March 22 and May 21, 1934, when the animal was found dead, it received fifty injections of pitressin. A large gastric ulcer was found in the pyloric part.

INTERPRETATION OF THE RESPONSE IN THE PITRESSIN EPISODE

The observations presented here definitely point to a conditioning of the blood vessels as the immediate cause of ulcer formation and

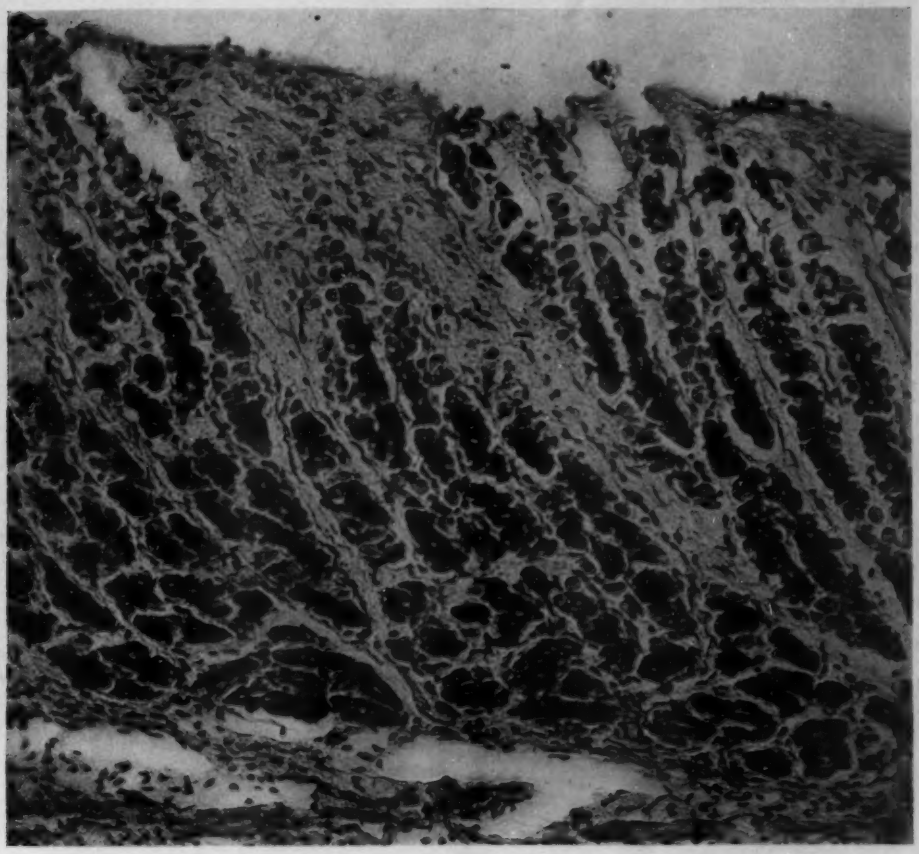

Fig. 14.—Surface scar; × 110. This is from the same dog as that in figure 13. It shows an old erosion filled with exudate partly organized. The superficial surface is covered with fibrinous exudate.

indicate that the "spasm theory" is the most logical to explain the periodicity in the appearance and disappearance of ulcers, as well as the fact that acute ulcers are frequently multiple and have a tendency to be widely and irregularly distributed in the stomach, as well as in the duodenum.

Pitressin injected intravenously evokes spasm of the small blood vessels and also spasm of the muscular tissues, which again compress the blood vessels. The contraction temporarily creates anoxia of the parenchymal cells, but later there follows dilatation of these blood vessels, the act of restoration. A normal biologic rhythm of this type keeps

Fig. 15.—Superficial erosion; × 90. The specimen is from dog 5, which received twenty-one injections of pitressin between March 3 and July 17, 1937, and was killed July 21. The ulceration on the surface extends to the muscularis submucosa.

vascular supply and demand in constant equilibrium, but the same contraction whether due to changes in the blood vessels or to contraction in the muscles (which compress the vessels) will, if prolonged, injure the parenchymal cells because it will be associated with undue general or local anoxia.

The greater the discrepancy between the demand for oxygen and the supply, the greater the changes which will follow, the range extending from purely functional changes, which cannot be discerned histologically, to necrosis of the tissue in different stages and of varying extent as here detailed. This has been shown in connection with the production of experimental endocarditis (Nedzel [6]) and in the stomach (Nedzel [9]) and has also been observed by me in the brain, liver and kidneys (unpublished observations).

With the pressor phase as it occurs under natural conditions of life (i. e., with cold, with relative alkalosis, with sympathicotonia, etc.) or

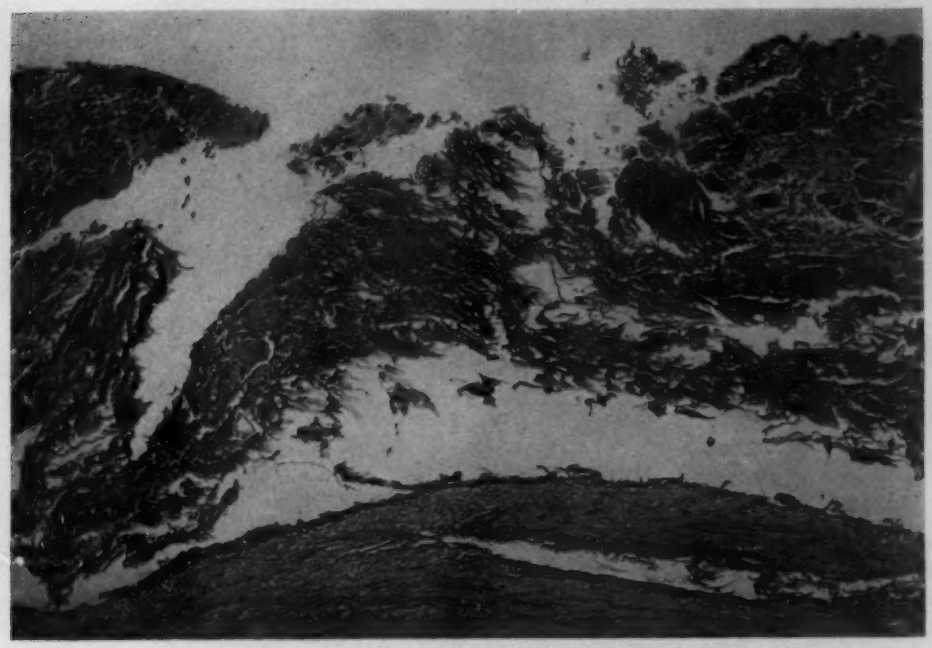

Fig. 16.—Ulcer; × 20. The specimen is from dog 6, which received thirty-five injections of pitressin between April 6 and July 25, 1934, and was killed July 25. This deep ulcer extends from the gastric surface through the submucosa and perforates the muscularis submucosa. Note serous exudate in the submucosa, necrosis of structures adjacent to the ulcer and edema of the muscular tissues.

after injections of pitressin, contractions of the blood vessels occur which may reach such an extent that a vessel filled with blood may rupture and establish a hemorrhage directly into the lumen of the stomach (fig. 6). In the wall of the stomach small hemorrhages and also foci of necrosis can be observed in the mucosa. In the submucosa and in the muscular layer dilated blood vessels filled with leukocytes, some of

which may appear perivascularly, can be discerned. On the surface of the mucosa an exudate may form, containing fibrin and formed elements which have passed through the undamaged epithelial layer. Erosion, edema of the wall of the stomach, necrosis of the mucosa, associated with an increased number of mononuclear cells, healing of the erosions and ultimately typical ulcer formation may also be seen.

SEASONAL CHARACTER OF THE RESPONSE

All these experiments have been carried out on healthy animals; the weak or sick were always discarded. It is of interest to note the seasons

Fig. 17.—Ulcer; × 20. The specimen is from dog 7, which received twelve injections of pitressin from Aug. 3 to Aug. 10, 1937, when the dog was killed. This is a chronic deep ulcer penetrating into the submucosal base formed by the muscular layer. Note a thrombotic blood vessel in the lesion at the left. The ulcer itself contains fibrous exudate with tissue débris.

in which the findings were positive. In September, October and November 15 dogs were given injections of pitressin, and in 20 per cent the findings were positive. In December, January and February 18 dogs were submitted to the experiment, and in 50 per cent the results were positive. In March, April and May 13 dogs were given injections, and in 61 per cent the findings were positive. In June, July and August, of 16 dogs, 23 per cent gave positive results.

It seems that normal animals which have been fatigued and are more acid and thus biologically weaker reveal greater autonomic difficulty in adjustment to meteorologic changes, and in them superimposed pressor effects from injections of pitressin apparently lead more readily to prolonged spasm and to prolonged recovery from the effect of spasm.

The ulcers of the stomach formed experimentally, as well as those established clinically, heal readily. The so-called chronic ulcers, of the type shown in figure 18, require an additional factor, also of nutritional character, and do not occur so commonly. The regularity with which peptic ulcers are found in certain persons leads some authors to speak

Fig. 18.—A large gastric ulcer in the pyloric part of the stomach of dog 8. This animal received fifty injections of pitressin between March 22 and May 21, 1934, when it was found dead.

of an ulcer diathesis, thus associating ulcers with definite constitutional trends in persons subject to this disease. It is generally agreed that ulcers are the result of metabolic disturbances, the local cause being chemicomechanical and the underlying one constitutional. But since the constitutional picture is quite overshadowed by the urgency of the local symptoms, the latter have led, and still do lead, to almost exclusive consideration of the ulcer rather than of the patient as a whole. But it is well recognized that persons subject to ulcer formation are usually

slender (asthenic persons) with labile nervous and vascular systems. The disease is seasonal and occurs mostly in the northern latitudes, and Petersen [10] recently showed that the exacerbations are connected with certain meteorologic changes.

The seasonal character of the disease can be related to the observations reported here. It is likely that these seasonal variations are in part connected with the systemic stimulation of the entire organism during the late winter and spring; at least there is a greater tendency toward inflammatory reaction and toward digestion of tissue. In the northern latitudes of the United States, meteorologic variability (shifting cyclonic circulation of the atmosphere with the passage of polar air masses), to which the human body has to adjust itself, is great, and the vascular adjustment implies prompt and marked alteration. The normal healthy person readjusts fully in due time, for his vascular system synchronizes readily, while the person with an unstable vascular system presents difficulty in equilibration. As a result episodes of undue vascular spasm are common. The action of pitressin is in a measure analogous to the action of meteorologic factors (pressor episodes) which one meets daily.

SUMMARY

Injections of pitressin readily produce gastric and duodenal ulcers in experimental animals (dogs), and these are located in places common to the same lesions in man. Direct observations of the mucosa when vascular interference in local nutrition occurs during and after spastic contraction of the muscles of the stomach and of the vascular coats of the blood vessels in the stomach and duodenum, together with histologic observations of the progression of ulceration from the very beginning to the end stages, definitely reveal the role of vascular dysfunction in ulcer formation. Mere functional spasm as produced in the experiments described, if prolonged and repeated, will lead to the development of ulcers in animals which normally do not suffer from such lesions. It has been shown that vascular interference with local nutrition is the primary factor in these pathologic developments. Depending on constitutional type, on vascular instability and on endocrine imbalance, on seasonal variation, on emotional situations, on trauma and on meteorologic factors, vascular incoordination may evidently be of sufficient intensity or duration to lead to prolonged local anoxia with resulting development of gastric and duodenal ulcers. Peptic ulcers should therefore be regarded as a local expression of a vascular dysfunction inherent in constitutional types unable to withstand the demands of the organism for adjustment to unusual environmental situations.

10. Petersen, W. F.: The Patient and the Weather, Ann Arbor, Mich., Edwards Brothers, Inc., 1935, vol. 2, p. 255; 1938, vol. 4, pt. 3, p. 131.

PATHOLOGIC OBSERVATIONS ON THE ADENOMATOUS LESION OF THE STOMACH IN MICE OF STRAIN I

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Spontaneous tumors of the stomach are rare in mice. Murray [1] described 2 cases of squamous cell carcinoma of the cardiac portion of the stomach, and Itami [2] reported a case of squamous cell carcinoma of the same region. Little and Tyzzer [3] observed a carcinoma in the stomach of a wild mouse which they diagnosed as arising from the squamous epithelium. Slye, Holmes and Wells [4] added 4 others; 3 of these they diagnosed as squamous cell carcinoma involving the cardiac portion, while the fourth they considered a primary tubular carcinoma. They summarized the literature up to 1917 as follows:

> Of the eight cases of carcinoma of the stomach of mice now recorded, seven are squamous cell carcinomas involving solely the cardiac portion, and only one corresponds to the tubular, cylindrical type of cancer found in man. Four occurred in females and four (including three of our four) were in males. Four showed metastases into the regional lymph nodes, one having in addition an ovarian metastasis.

These same authors reported another case of tumor involving the stomach of which a correct diagnosis could not be made. In certain respects it resembled "a small round cell sarcoma arising in the wall of the stomach . . . but it is impossible to say whether the growth arose primarily in the stomach or in the mesenteric lymph nodes."

Bonne,[5] during the course of a series of tar-painting experiments, kept 146 mice as untreated controls and in 2 of these he found papillomas of the forestomach. The animals involved were between 7 and

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From the Office of Cancer Investigations, U. S. Public Health Service, Harvard University Medical School.

1. Murray, J. A., in Bashford, E. F.: Third Scientific Report on the Investigations of the Imperial Cancer Research Fund, London, Taylor & Francis, 1908, p. 71.

2. Itami, S.: Proc. New York Path. Soc. **16**:170, 1916.

3. Little, C. C., and Tyzzer, E. E.: J. M. Research **33**:396, 1916.

4. Slye, M.; Holmes, H. F., and Wells, H. G.: J. Cancer Research **2**:401, 1917.

5. Bonne, C.: Ztschr. f. Krebsforsch. **25**:1, 1927.

12 months of age. Mercier and Gosselin [6] found a papillary carcinoma in the forestomach of a male mouse following intraperitoneal injection of tar in olive oil, which they regarded as resulting from ingestion of hair contaminated with tar. These authors reported another growth, a lymphosarcoma, found in the stomach of a female mouse with generalized lymphosarcoma. It was impossible to tell whether the tumor of the stomach was primary or secondary.

Bittner,[7] in a publication dealing with the influence of cross breeding on the incidence of spontaneous cancer of the breast in mice, mentioned that 2 females of the I strain, one of which was 19½ months of age, died with "adenoma of the stomach." This report is of considerable interest as regards the present communication, because the adenomas were found in mice of strain I.

Mice of strain I have been raised in this laboratory since 1933. All are descendents of one litter procured from the Roscoe B. Jackson Memorial Laboratory. As stated previously (Andervont and Stewart [8]), the strain was originated by Dr. L. C. Strong in 1927 by crossing a pink-eye dilute brown and a dilute piebald stock. Five recessive characters were selected from this cross, namely: pink-eye, dilute, brown, nonagouti and piebald, and the strain continued by brother to sister mating. A spontaneous adenomatous lesion of the pyloric chamber of the stomach develops in all mice of the I strain except those dying early in life.

MATERIALS AND METHODS

Twenty-seven untreated mice of this strain aged 2.5 to 16 months were killed and autopsies made. There were 5 males and 22 females. All animals raised in this laboratory are maintained on a diet of Purina dog chow [9] exclusively, water being allowed freely. The stomach specimens were preserved by different methods: A few were opened and spread on a cork; others were dropped into fixative immediately when removed from the body; in approximately half the cases the viscus was first ligated at both orifices and then distended with fixative. A few animals which died spontaneously were excluded from histologic consideration owing to the possible presence of postmortem autolysis. Two control mice from each of the strains Y, Robert's pink eye, A, D and C57 black, aged respectively 11 and 11½, 12 and 17, 12 and 12½, 12 and 12, and 16 and 16 months, were killed and examined. The tissues for histologic study were fixed in 4 per cent solution of formaldehyde and Tellyesniczky's, Zenker's and Bouin's fluids. Sections of stomach were cut in several different planes and stained with hematoxylin and eosin, Masson's trichrome stain, eosin-methylene blue and Mallory's aniline blue connective tissue stain.

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6. Mercier, L., and Gosselin, L.: Compt. rend. Soc. de biol. **113**:669, 1933.

7. Bittner, J. J.: J. Hered. **27**:391, 1936.

8. Andervont, H. B., and Stewart, H. L.: Science **86**:566, 1937.

9. According to the formula supplied by the Purina Mills Company the chow contains the following substances in the percentages given: fat, 5; protein, 4; fiber, 7; ash, 7; moisture, 7, and nitrogen-free extract, 54.

ANATOMY AND HISTOLOGY OF THE STOMACH OF THE NORMAL MOUSE

The stomach of the normal mouse is divided into two portions, separated by a sharp line which is easily discernible on both internal and external surfaces of the organ. The left or cardiac two thirds is lined by squamous epithelium directly continuous with that of the esophagus and is comparable to the rumen of the herbivorous animal. The pyloric chamber, much smaller in extent and forming one third of the whole organ, is lined by glandular epithelium similar to that of other mammals and terminates in the duodenum. In the present control mice no significant changes were observed in the autopsies made. The stomachs were thin, smooth and soft and contained varying amounts of food; the glands were regular and not dilated. Foci of inflammatory cells and one or two fragments of squamous epithelium were occasionally found below the surface mucosa at the esophageal and midgastric junctions, respectively, but these observations were regarded as falling within normal limits. However, in studying routine sections of tumor-bearing mice on which autopsies had been made in these laboratories during the past several years a somewhat similar but much less extensive lesion than that occurring in mice of strain I, as described later, was encountered in 3 mice of strain D, 2 of strain C57 black, 2 commercial mice and 3 mice of strain C_3H, all of which were in the older age groups.

MORPHOLOGIC OBSERVATIONS

The adenomatous gastric lesion in the I strain of mice, although making its appearance relatively early in life, did not become pronounced until the animals were from 8 to 10 months of age. Thus, of 3 mice aged 2½ months, a single mouse had in the gastric glandular mucosa a few slightly hyperplastic areas on either side of the esophageal orifice. Ordinarily such observations would be passed by as of little significance, but in view of the subsequent events known to occur in this strain of mice, these early changes are viewed as the origin of the adenomatous gastric process. The stomach specimens from the other 2 young mice were thin walled and perfectly smooth, with normally proportioned cardiac and pyloric chambers, sharply demarcated from each other and distended with recently ingested food. In mice between the ages of 2½ and 6 months no lesions have as yet been studied, but by 6 months the pyloric chamber had become enlarged and thickened. Areas in the glandular mucosa showed beginning abnormal appearances in the glandular cells and acini, between which, by this time, were interspersed collections of inflammatory cells. From this time on the lesion developed progressively.

The gastric overgrowth reached its peak after the mice were 8 months of age. Twenty-one animals in this group were examined post

mortem. During this period some change was present in all specimens examined, the lesion being usually pronounced and in most instances relatively uniform in degree and extent of involvement.

It was not uncommon to detect the lesion by inspection of the living animal, since the stomach was of sufficient size to produce a definite swelling of the abdomen. When the peritoneal cavity was opened, the stomach regularly lay horizontally high in the abdomen, well up under the diaphragm, and usually completely concealed by the liver except for a small area at the inferior border. The serosa was congested. The cardiac chamber was wrinkled and compressed against the costal cage. It contained little food. Its inner lining was usually thin and transparent and occasionally roughened. These roughened areas in the cardia were due to slight hypertrophy and hyperkeratosis of the squamous epithelium. The pyloric chamber formed the main body of the viscus and was solid, firm and opaque, with gray nodular or ridgelike corrugations on the external surface. Occasionally small pouchlike diverticulae projected beneath the serosa. The glanular mucous membrane of the pyloric chamber was the seat of pronounced thickening and polypoid formation. The lumen of this portion of the stomach was considerably reduced in caliber and contained little or no food (fig. 1*A*). The adenomatous areas of involvement were symmetrically distributed in the gastric mucosa. The lesion began abruptly at the junction of the two chambers, partially encircled the esophageal opening and spread along both curvatures over the entire inner circumference of the proximal portion of the chamber, fading out gradually toward the duodenal orifice (fig. 1*B*). The longest spread of the lesion occurred along the greater curvature. In the area of involvement the mucosa appeared thick and rough with coarse, hypertrophied rugae capped by gray excrescenses (fig. 1*C*). In a few specimens the gastric lumen was chiefly occupied by flat nodular masses, leaving only a narrow tortuous channel for passage of food and other contents. No extensive necrosis or sloughing of the affected mucous membrane was observed.

Microscopically, the process responsible for these gross changes consisted in an adenomatous, hypertrophic, hyperplastic overgrowth of the glandular rugae of the pyloric mucosa (fig. 2*A* and *B*); degenerative and inflammatory changes were present as well as a development of atypical epithelium (fig. 2*C* and *D*) with limited penetration into the deeper portions of the gastric wall and blood vessels (fig. 3*A* and *B*). The lesion began abruptly in the glanular mucous membrane along the line of constriction encircling the middle of the stomach. As observed in serial cross sections of the distended, fixed viscus, the lining membrane became thickened, forming an abrupt triangular peak within the

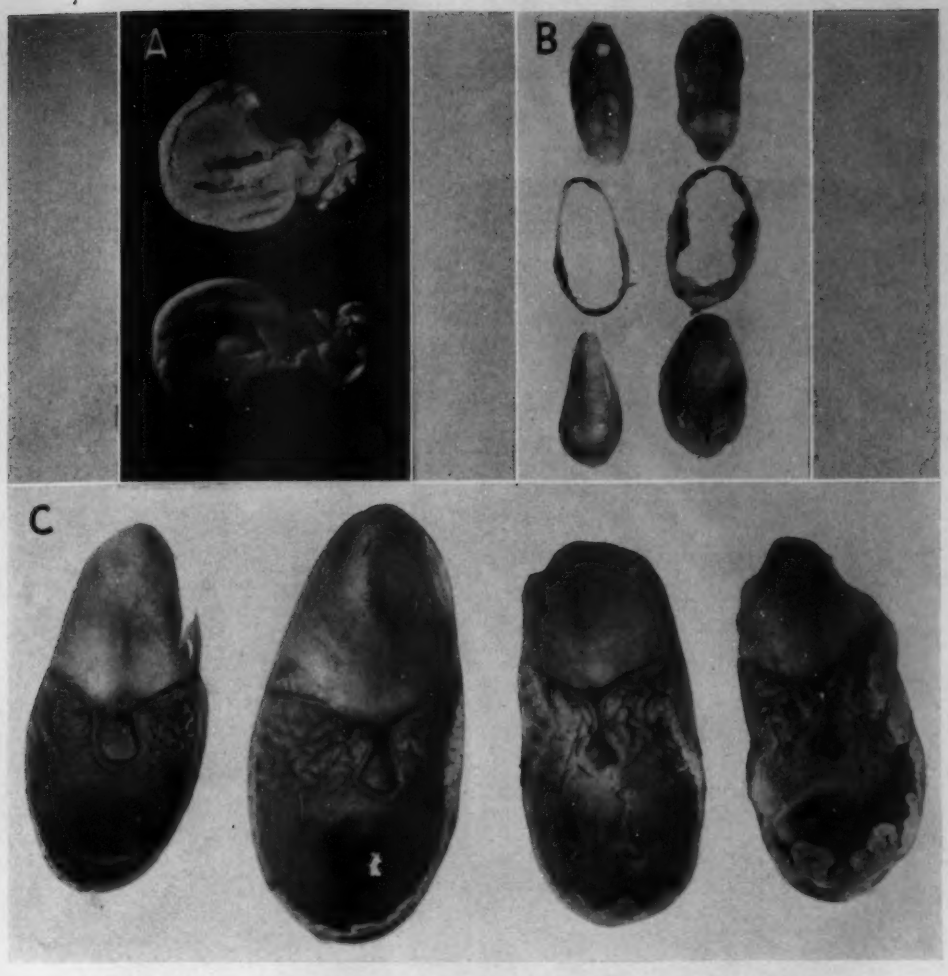

Fig. 1.—*A*, stomach of a male mouse of strain I, aged 10 months. At autopsy the organ was removed from the body, immediately hardened in fixative and later split in half longitudinally. The lumen is occupied by large nodular projections of the hypertrophied mucous membrane, leaving only a narrow tortuous channel for the passage of food and other contents. The cardiac chamber is small and compressed.

B, two stomachs each cut in three pieces. Each specimen was prepared by injecting fixative into the lumen at autopsy and allowing it to harden for twenty-four hours before sectioning. The specimen on the left is from a female mouse of strain D, aged 12 months. The smooth portion of the viscus represents the squamous cell–lined cardiac chamber, and the rough portion, the glandular mucosa of the pyloric chamber. This may be designated as the stomach of a normal mouse. The specimen on the right is from a female mouse of strain I, aged 11 months. Note the polypoid thickening of the glandular mucosa and the hypertrophy of the pyloric chamber. The specimens are shown slightly reduced.

C, superior halves of four stomach specimens from female mice of strain I, aged from left to right 2½, 6, 10 and 11 months. Note the symmetric distribution and progressive development of the glandular thickening of the pyloric chamber with age. The upper part of each specimen is the cardiac portion. In the two specimens on the right, the squamous lining of the cardia is also slightly roughened, and the esophageal orifice which lies at the junction of the chambers is partially obstructed. Magnification, × 1.9.

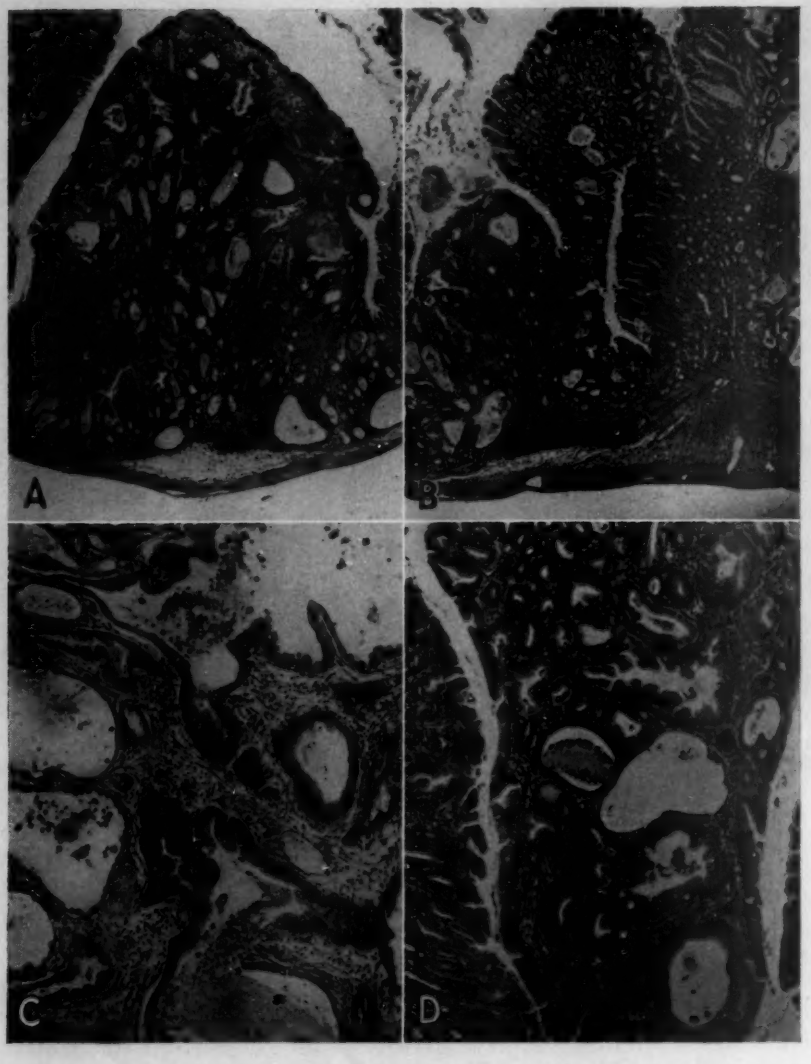

Fig. 2.—*A* and *B*, areas of the glandular mucosa of the stomachs of 2 mice of strain I, one a female aged 11 months and the other a female aged 10¼ months. The mucosa is thickened by hypertrophied rugae composed of atypical glands, some of which indent the basement membrane. Note also the inflammatory changes. Magnification, × 32.8.

C and *D*, areas in the glandular mucosa of the stomachs of 2 mice of strain I, a female aged 10¼ months and a male aged 11 months. Note the atypical glandular structure, hyperplasia, metaplasia and desquamation of epithelial cells and inflammatory changes in the stroma. Magnification, × 65.7.

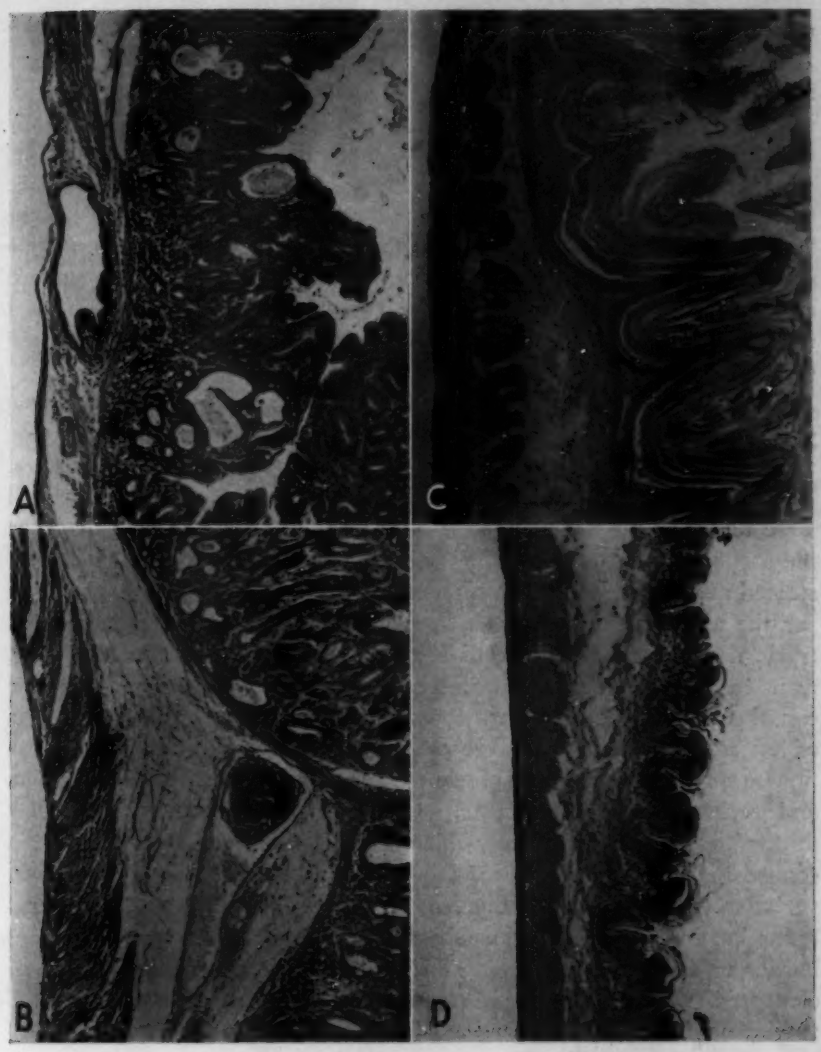

Fig. 3.—*A* and *B*, stomachs of 2 mice of strain I, a female aged 10 months and a male aged 11 months. Note the solitary glandular fragments in the muscular wall in *A* and in a vessel well below the basement membrane in *B*. In *A* note the fold in the muscular coat on each side of the glandular fragment. Magnification, × 65.7.

C and *D*, cardiac chambers of the stomachs of 2 mice. In *C* note the thickening and hyperkeratosis of the squamous mucosa in the stomach of a female mouse of strain I, aged 15 months, as contrasted with the cardia of a female mouse of strain C57 Black, aged 16 months, shown in *D*. Magnification, × 65.7.

first centimeter of its origin and then gradually receding toward the duodenal orifice (fig. 1*B* and *C*). The hyperplastic change was almost completely limited to the gastric glandular mucosa as described, with relatively slight involvement of the pyloric valve and antrum. Occasionally Brunner's glands in the duodenum were interspersed with irregularly dilated acini, but the overlying mucosa was practically unaltered. Likewise the squamous epithelial lining of the cardiac chamber was usually unchanged, but in a few specimens from old mice there were hypertrophy and hyperkeratosis, which caused slight roughening of the surface as illustrated in figure 3*C*. Sometimes one or two masses of squamous epithelium were present below the surface at the mucosal junction between the two chambers, but as this observation was also made in the control material no significance was attached to it.

Examining a section of the gastric mucous membrane along the greater curvature, starting at the duodenal orifice and working proximally, one noted a gradual change within the first several millimeters. The mucosa gradually became interspersed with atypical tubules, which were not very numerous at first but which increased progressively as the section was moved along. These tubules were coarse, often deeply stained and varied considerably in caliber, with little tendency, however, for the atypical epithelial cells to grow in solid masses. On the contrary, nearly all the living cells seemed securely anchored to the basement membrane, and the lumens of the glands were either empty or contained dead cells and other nonviable material. The thickened areas of the mucous membrane were composed of polypoid glandular elevations, representing modified rugae supported on connective tissue stalks and separated by deep crypts. Contributing also to the glandular thickening were the markedly dilated acini. The glandular structures comprising these polypoid projections showed considerable morphologic variation. Dilated acini occurred at all levels of the mucous membrane but in certain specimens showed a predilection for the superficial border or for the deep layer. These structures were round, oval and elongated, with the long axis running in various directions. In some specimens there was a formation of microcysts with spurs and reduplication of the lining cells and hyperplastic tubular glands which traversed a tortuous course toward the surface. The lining cells of these distended glands were often piled up two or more layers thick, imparting a saw-tooth appearance.

Coinciding with the onset of change in structure in the gastric glands, alteration occurred in the normal arrangement and appearance of their lining cells, which differed considerably in size, shape and staining, with three chief types predominating: 1. A columnar cell was observed resembling that normally present but often hypertrophic and

distorted, with an oval, basally situated nucleus staining variably from light to deep basophilic and surrounded by dark granular cytoplasm with occasionally single large vacuoles near the free border. Many nuclei were hyperchromatic. Instead of maintaining their perpendicular position with respect to the basement membrane, the cells were often tilted at angles, some being end to end or parallel. 2. The second type of cell was represented by a large foamy cuboidal or polygonal cell of varying shades, slightly acidophilic, with a round pale nucleus containing fine chromatin particles. The free borders of neighboring cells lining acini often differed in height, which, together with miniature papillary excrescences, composed of clumps of from 5 to 7 cells, resulted in irregularity in outline and lack of definition of the glands. 3. Finally there were metaplastic flattened cells which varied from thick to extremely flattened, elongated squamous forms with pink granular cytoplasm and pale nuclei, proportional in size to that of the cell. Necrosis, desquamation and keratinization were frequently noted. At all levels of the mucous membrane mitotic figures and transition stages between these various types of cells were observed, and large acini contained all representatives, including intermediate forms.

Most acini were empty. A few contained stringy mucin, desquamated cells or keratin. In one specimen the edge of the mucosa bordering either side of a deep crypt showed a colloid type of degeneration, apparently originating in the stroma and involving the glands secondarily. In other specimens the connective tissue at the terminal tips of many of the villous stalks was pale and edematous. The stroma was congested, infiltrated with inflammatory cells and generally sparse and fine. Inflammatory cells, chiefly leukocytes, infiltrated deeply at the junction between the two chambers and less densely in various areas in the mucosa and muscle, which showed small foci of destruction and necrosis.

The usual careful search for metastasis at autopsy has so far yielded completely negative results; special attention was directed toward the ordinary routes of spread of gastric cancer. Although a number of animals had pulmonary nodules, these were not regarded as metastases, since they corresponded morphologically with the common primary tumor of the lung which is frequently observed in certain strains of mice. Furthermore, these spontaneous nodules were structurally identical with the pulmonary tumors induced by treating these mice with carcinogenic hydrocarbons.

In view of the marked deviation from the normal appearance of the glandular mucosa, together with the progressive character of the lesion as judged by the gross appearance of the stomachs of the I mice of different ages, the possibility of malignant degeneration received careful consideration. In this connection certain appearances were noted in the histologic sections which were not fully in accord with the usual con-

ception of an entirely benign lesion. On the other hand, the areas of glandular hyperplasia were symmetric in their distribution in the gastric mucosa, as shown in the illustrations, and in no instance was there an unlimited infiltration of atypical epithelium into the wall of the stomach or widespread destruction as commonly occurs in human gastric cancer. However, in the histologic sections of the stomachs of the mice of strain I it was not unusual to see a number of the more dilated, deeply situated acini indenting the basement membrane of the mucosa. furthermore, in at least a third of the cases solitary fragments from the altered mucosa lay well below the basement membrane apparently completely isolated from the surface. These deep extensions consisted in most instances of not more than one or two glandular fragments per section lying in the submucosa, muscle or beneath the serosa (fig. 3*A*). Intravascular extension occurred in two specimens, one of which is illustrated in figure 3*B*, showing a glandular fragment occupying the lumen of a vessel. Although it was fairly common for distended acini to be seen indenting the basement membrane, yet comparatively few of these infiltrated the deeper wall of the stomach or the blood vessels. In the stomach specimens harboring these deeply placed glandular fragments one could often recognize a local fold in the basement membrane or muscular coat suggesting a persisting connection between the fragment and the surface mucous membrane. This point is at present under investigation.

As the animals grew old and the adenomatous glandular overgrowth in the stomach advanced, pronounced anemia developed, which was often proportional in degree to the severity of the gastric process. The figure usually accepted for the red blood cell count of mice is about 9,000,000 to 10,000,000. Red blood cell counts above 12,000,000 have been obtained in mice of strain I. Ultimately, however, in these animals there was a drop in the red cells, sometimes to 20 per cent or less of the normal. Coincident with this numerical decrease a marked morphologic change occurred in the erythrocytic series. The stained smears showed achromia, polychromasia anisocytosis, poikilocytosis, macrocytes, microcytes, stippling and an occasional Howell Jolly body and an occasional normoblast. A group of 5 male mice aged 9 months all showed anemia, the degrees of which could be roughly correlated with the severity of the gastric lesions. Thus the animal in which the stomach was least affected showed only slight anemia, whereas the red cell count in the remainder was below 4,000,000, and in a mouse which was moribund and showed at autopsy a pronounced gastric lesion the count was below 2,000,000. Sometimes an active, vigorous, apparently healthy animal dies suddenly, and the autopsy reveals massive gastrointestinal hemorrhage extending into the first few coils of the small intestine. This type of death is rare, however, and it is more common

to find the animals becoming listless over a period of weeks, gradually losing weight, becoming anemic and finally dying. It is surprising to note that the animals often eat until within an hour or so of death.

No infectious organism has been constantly demonstrated to be associated with the lesion developing in the stomach of the mouse of strain I. At autopsy the viscus has been carefully inspected for gross infestation by worms and other large parasitic forms, but none has been found. In several instances small clusters of yeastlike organisms and various bacterial forms were observed on the surface of the fixed and stained stomach and in the superficial crypts of the mucosa, but similar organisms were also present in the control material. In two specimens microorganisms resembling the schizont forms of the protozoon parasite Klosiella muris (Wenyon [10]) were detected in the blood vessels in the hyperplastic mucosa, sometimes occupying swollen endothelial cells, but since protozoa have been consistently absent from all other gastric specimens, their importance in this connection is as yet undetermined.

COMMENT

The degree and extent of involvement of the stomach by the gastric lesions under consideration are fairly comparable in animals of the same age and sex. However, the examination of the 5 male animals mentioned in this communication and others from various investigations suggested that the lesion may appear earlier and be more pronounced in male than in female mice. In order to investigate this possibility a number of animals of both sexes have been set aside and are being used to determine more precisely the development of the lesion. The change is progressive and develops symmetrically in the stomach, reaching its peak of severity after the eighth month of life. Although differences in appearances of individual stomach specimens are detected up to 16 months of age, yet all animals are affected by late middle life. Mice of strain I are very active normally, but after the gastric lesion has become more advanced they show a change in their behavior by becoming less active and huddling together in the case, with their hair ruffled and their bodies humped. The skin of their paws and the mucous membranes turn gray, and their blood becomes pale and watery. These appearances are due to pronounced anemia, the red blood cell count not infrequently dropping to 20 per cent of normal or even less. The various features of this anemia, such as time of onset, rapidity of development, response to various therapeutic measures and relation to structural changes in the bone marrow, are at present under investigation. Future investigations of the lesion in mice of strain I may open

10. Wenyon, C. M.: Protozoology, New York, William Wood & Company, 1926, vol. 2.

up avenues for studies which may have some bearing on the appearance of gastric lesions or associated manifestations in other species.

The true nature of the gastric process of the mice of strain I is not clear. Histologically, it is classified as an adenomatous overgrowth of the glandular mucosa. However, in considering the profound alteration of tissue that may follow nutritional disturbances, too strong a stand for the neoplastic character of the lesion under consideration does not seem warranted. Cramer [11] has written an excellent summary and has presented new information concerning the benign hyperplasia and hyperkeratosis of the squamous epithelium of the rat's forestomach resulting from unbalanced diets and other factors. The question of diet as an etiologic factor in mice of strain I is at present under investigation. So far no single organism, large or small, has been consistently observed in the sections of the stomach. However, the possibility of an infectious etiologic agent cannot be entirely dismissed from consideration since there is regularly an infiltration of inflammatory cells in all coats of the stomach exhibiting the adenomatous change. Minced stomachs from old mice of strain I have been fed to mice of other strains, and mice of strain I have been quartered in the same cages with other mice in order that this possibility might be investigated. These experiments are now in progress.

Somewhat suggestive of malignant growth was the replacement of the gastric glandular mucosa by a complex arrangement of glandlike structures composed of altered epithelium, with infiltration of atypical acini into the deeper part of the wall and in 2 cases by permeation of veins. Although in clinical material such findings are considered highly suggestive of malignant change, it would be premature to make the same interpretation in the case of the mouse. First of all, the deep extensions of glandular fragments into the gastric wall as illustrated were well limited and not disorderly as in clinical cancer, and there was frequently a suggestion of contact between the surface mucosa and the deeply placed fragment through a fold in the wall of the stomach. Although large irregular acini indented the basement membrane in many places, only one or two atypical epithelial fragments gained access to the deeper tissues in any specimen. The glandular mucosa was roughened and wrinkled but freely movable on the underlying muscle, which is in sharp contrast to the condition of linitis plastica, with which the process may be contrasted and in which all layers of the stomach are rendered immobile. In the next place, metastases have not been observed in any of our material. As stated, a few adenomatous tumors were found in the lungs of some mice, but since these corresponded

11. Cramer, W.: Am. J. Cancer **31**:537, 1937.

in all respects to the common pulmonary tumor that is characteristic of this species there is no reason for regarding them as embolic deposits from the stomach. As further attesting to their nonmetastatic nature, these pulmonary growths appeared in increasing numbers following injection into I mice of 1,2,5,6-dibenzanthracene, corresponding in this respect to the pulmonary tumors of strain A and other mice. Finally, the gastric process in the I mice develops remarkably symmetrically, all lesions conforming to the same general pattern. Instead of a single tumor arising in a different portion of the stomach in the various specimens, the lesion reduplicates itself from mouse to mouse with marked exactness, as may be observed when sections are obtained from the same planes in any series of cases. In the stomachs it is the same areas of all the animals that undergo the adenomatous proliferation; the sites of localization from mouse to mouse are remarkably uniform. All stomachs from the I mice conform to the same general pattern. A dense resistant mass forms along the immediate line of origin of the glandular mucosa and diminishes in extent toward the duodenal orifice, making the pyloric chamber thick and rigid. Locally there is a suggestive resemblance to a leather bottle stomach, and the secondary physiologic effects on the organism may be much the same. However, in contrast with the leather bottle stomach of man, the adenomatous change in the I mice is superficial and chiefly limited to the mucosa.

As stated previously, a lesion resembling that seen in the stomachs of mice of strain I has been observed occasionally in mice of other inbred strains. This suggests that the occurrence of the lesion in the I mice might be analogous to the occurrence of spontaneous pulmonary growths in certain inbred strains of mice, for it is known (Andervont [12]) that some strains have a high incidence of spontaneous pulmonary tumors while others have an extremely low incidence of the same type of growth. Furthermore, it is known that in mice susceptibility to spontaneous pulmonary tumors is inherited according to genetic principles, and it will be of interest to see if future investigations reveal a genetic influence in the transmission of susceptibility to the stomach lesion.

The effects of the adenomatous gastric overgrowth on the organism of the mouse are those of progressive anemia, loss of activity and hemorrhage. When death is spontaneous late in life, little or no food is found in the stomach at autopsy. Although the lumen of the viscus is gradually narrowed by the overgrown mucosa, there does not appear to be any dilatation of the alimentary canal proximal to the lesion. On the contrary, the cardiac chamber of the stomach is usually compressed and small, and the esophagus is not frequently widened, thus arguing

12. Andervont, H. B.: Pub. Health Rep. **52**:304, 1937.

against stagnation of ingested material or interference with motor activity in propelling food through the narrow channel of the stomach. Loss of weight is not as striking as might be supposed. Practically none of the animals that die show the extreme emaciation that often accompanies spontaneous or induced subcutaneous tumors or other debilitating conditions.

Finally it should be pointed out that the lesion in the stomachs of the I mice bears some analogy to a rare gastric condition occurring in man which has been reported under various titles, such as "gastritis polyposa," "adenomatosis gastrica" and "adenopapillomatosis gastrica." The pathologic picture is well described by these terms, and those who have had personal experience with the disease consider that it may originate in either of two ways: (1) as congenital hypertrophy of the epithelium; (2) as hyperplasia acquired through chronic inflammation. One writer claims to have demonstrated all stages from hypertrophic gastritis to adenomatous formation and even to carcinomatous change (Konjetzny [13]). There is evidence that a congenital factor may be present. The disease has been found in twins, in brothers and sisters and in mother and son, and in 7 per cent of a series of cases collected by Brunn and Pearl [14] it was associated with generalized polyposis of the intestinal tract, a condition which shows a distinct familial tendency.

SUMMARY

A spontaneous adenomatous lesion in the pyloric chamber of the stomach in mice of strain I is described and illustrated. The lesion occurs in virtually all mice of both sexes of this strain and is the chief cause of death in mice of this strain. Mice of strain I are known to die at a relatively early age. The lesion is characterized by an adenomatous, hypertrophic, hyperplastic overgrowth of the glandular rugae of the pyloric mucosa; degeneration and infiltration by inflammatory cells were present together with development of atypical epithelium with limited penetration into the deeper gastric wall and blood vessels. Although histologic studies reveal certain features that are somewhat suggestive of malignant growth, there are contradictory criteria, such as the symmetric development of the process and the absence of metastases, unlimited local spread, extensive ulceration and destruction.

13. Konjetzny, quoted by Sinclair, N.: Brit. J. Surg. **20**:645, 1933.
14. Brunn, H., and Pearl, F.: Surg., Gynec. & Obst. **43**:559, 1926.

CIRRHOSIS OF THE LIVER IN RABBITS WITH CONTINUED CHLOROFORM POISONING AND WITH ASSOCIATED SYPHILITIC INFECTION

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Cirrhosis of the liver has been produced in animals by means of long-continued repeated administration of chloroform by numerous investigators. Opie [1] found that the increase of connective tissue was more pronounced in those animals which also receive intravenous injections of colon bacilli or streptococci. An association, in man, of Laënnec's (nodular) cirrhosis with syphilis has been suggested by a number of observers including Symmers,[2] Owen,[3] LeDuc [4] and more recently Schumacher.[5] A relation between cirrhosis, syphilis and excessive use of alcohol has also been pointed out. In view of these observations, it was thought that administration of chloroform to rabbits infected with syphilis might produce an increased incidence of cirrhosis. Advanced nodular cirrhosis with ascites followed continued administration of chloroform, but there was no evidence that the lesion had been modified by syphilis.

EXPERIMENTAL PROCEDURE

Chinchilla rabbits were kept on a standard diet of oatmeal, bread and hay, with greens twice a week. Syphilis was produced by means of an intratesticular inoculation of an emulsion prepared from a chancre of the testis of a rabbit infected with the Nichols strain of Spirochaeta pallida, cultures of which were obtained through the aid of Dr. Louise Pearce, of the Rockefeller Institute for Medical Research, New York. At the sites of injection in the inoculated rabbits (except 3 which died of the initial dose of chloroform) primary lesions developed in from twenty-eight to fifty-five days. A number of the animals presented metastatic orchitis and a few keratitis, but other secondary lesions were not observed.

Chloroform was given subcutaneously every other week, in a quantity varying with the weight of the rabbits, commencing twenty-four or forty-eight hours before the introduction of spirochetes. It was mixed with an equal part of paraffin oil. Twenty-five hundredths cubic centimeter of chloroform U. S. P. per kilogram produced central necrosis of lobules of the liver but was not fatal. This amount

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From the Department of Pathology, Cornell University Medical College.

1. Opie, E. L.: J. Exper. Med. **12**:367, 1910.
2. Symmers, D.: Internat. Clin. **1**:58, 1917.
3. Owen, L. J.: Am. J. Syph. **5**:20, 1921.
4. LeDuc, D. M.: Ann. Int. Med. **2**:932, 1929.
5. Schumacher, G. A.: Am. J. M. Sc. **5**:693, 1937.

was given in the repeated injections after an initial dose of 0.5, 1 or 2 cc. per kilogram. In all, 37 animals were used. Of these, 16 (group A) were infected with S. pallida and received chloroform, 15 (group B) were given only chloroform, and 6 (group C) were given an injection of spirochetes alone. Of these animals, 10 of the first group (A) received only one injection of chloroform. One of the second group (B) received only two injections of chloroform and 7 of this group (B) only one injection. Twelve of the rabbits died from the initial administration of chloroform. None of the remaining 6 animals receiving only one injection showed any fibrosis of the central parts of the lobules, and these rabbits need not be considered further at this point. Of the 13 rabbits that received three or more injections of chloroform, 6 were infected with syphilis, while 7 were not. Among the 6 syphilitic animals, 4 presented central fibrosis. Of the 7 nonsyphilitic rabbits, 4 also showed central fibrosis. The extent of this cirrhosis is graded on the basis of 1 to 4 plus; a doubtful lesion is designated plus-minus (table). There is an insignificant difference in the percentage of cirrhosis in the two

Rabbits Receiving Three or More Injections of Chloroform

Experimental Group	Rabbit	Duration of Experiment, Months	Injections	Respiratory Infection	Central Fibrosis	Associated Lesions
A. Rabbits given chloroform and inoculated with S. pallida	11	8	18	0	++++	0
	30	2	5	+	++	0
	36	5½	10	+	++	Metastatic orchitis; keratitis
	37	1	3	0	+	0
	35	5	8	+	0	0
	33	5½	10	0	0	Metastatic orchitis
B. Rabbits given chloroform alone	13	8	18	0	+++	
	22	5	8	+	±	
	24	3	7	+	++	
	29	1½	4	+	+++	
	25	5½	10	+	0	
	27	5½	10	0	0	
	28	5½	10	0	0	

groups (among syphilitic, 66.5 per cent; among nonsyphilitic, 49.1 per cent). In other words, repeated damage to the liver by chloroform in syphilitic rabbits did not materially increase the incidence of cirrhosis over that produced by chloroform alone.

In the course of the experiment there was an epidemic of the respiratory disease of rabbits known as snuffles, and the observation is noteworthy that of the 8 animals of both groups A and B which had central fibrosis of the hepatic lobules, 5 (62.5 per cent) had clinical or morphologic evidence of this infection, while only 25 per cent of the rabbits without fibrosis were infected. This suggests the possibility that the respiratory infection may have played a role in the production of the cirrhosis through direct bacterial action or through the influence of toxins. This suggestion is, however, not supported by the observation that the rabbits, one with syphilis and one without, which received the largest number of injections of chloroform (eighteen) and had the most advanced cirrhosis did not have snuffles.

Of the 6 syphilitic rabbits which received no chloroform, none had central fibrosis.

In the whole series of 37 animals there were 8 in which gross lesions of coccidiosis were recognized, but this disease was not present in the rabbits with

central cirrhosis. A mild degree of periportal fibrosis or of periportal lymphocytic infiltration or of both was seen in 13 animals; it occurred in 2 of the animals which had central fibrosis. This change was seen in animals which did not have the respiratory infection as well as in those which had it; it was less frequent in the rabbits that were infected with syphilis and received no chloroform.

COMMENT

Repeated injections of chloroform can produce in rabbits a cirrhosis of the liver which when advanced is nodular in character (fig. 1 *B*). The fibrosis in these experiments was central in the earlier lesions (fig. 2 *A*) and consisted of a condensation of the stroma followed by an increase. Later the periportal tissue was involved so that the central and peripheral zones became fused, and in the liver with the most advanced changes (rabbit no. 11, fig. 2 *B*) the lobular architecture was so distorted that it was difficult in places to distingush central from portal areas. Small bile ducts were increased in both places, and new nodules of hepatic tissue were formed. Mitotic figures were seen. In the peritoneal cavity of rabbit 11 was about 25 cc. of clear yellow fluid, while about 50 cc. of similar fluid was in the peritoneal cavity of rabbit 13.

Mertens, quoted by Moon,[6] gave chloroform mixed with paraffin to rabbits in doses of from 0.25 to 0.5 cc., at intervals of from three to ten days over a period of from six days to three and a half months. He produced cirrhosis which he claimed presented all the characteristics of Laënnec's granular atrophy of the liver in man. Schultz, Hall and Baker[7] injected chloroform directly into the portal vein and produced lesions resembling infarcts, followed by formation of granulation tissue with fibrosis. The lesions were irregularly distributed in the liver but affected chiefly the periportal parts of the lobule. With hypertrophy and hyperplasia of the liver cells, nodules were formed which imparted to the liver a granular or hobnailed appearance. Opie,[1] working with dogs, and de Zalka,[8] with rabbits, have obtained with chloroform fibrosis predominantly in the central parts of the lobules.

Lacquet[9] produced cirrhosis of the liver in white rats by repeated injections of carbon tetrachloride, a drug which, like chloroform, produces central necrosis of the lobules of the liver. Fibrosis disturbed the usual lobulation, dividing the parenchyma into separate and distinct small islands or pseudolobules. The organs were hard, with hobnail roughness of their surfaces. Cameron and Karunaratne[10] obtained

6. Moon, V. H.: Arch. Path. **18**:381, 1934.
7. Schultz, E. W.; Hall, E. M., and Baker, H. V.: J. M. Research **44**: 207, 1923.
8. de Zalka, E.: Am. J. Path. **2**:167, 1926.
9. Lacquet, A. M.: Arch. Path. **14**:164, 1932.
10. Cameron, J. R., and Karunaratne, W. A. E.: J. Path. & Bact. **42**:1, 1936.

periportal fibrosis in white rats with repeated injections of carbon tetrachloride. They expressed the belief that fibrosis in the central part of the lobule is the result of collapse of the hepatic structure and condensation of connective tissue and that it is incidental to the portal changes.

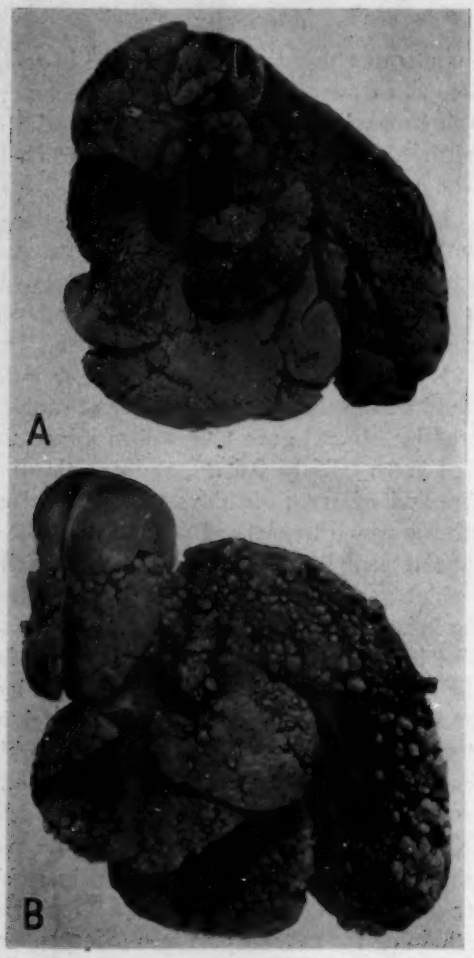

Fig. 1.—*A*, cirrhosis of the liver of rabbit 13 following eighteen subcutaneous injections of chloroform during a period of eight months. This animal did not have syphilis. *B*, nodular cirrhosis of the liver of rabbit 11 following eighteen subcutaneous injections of chloroform during a period of eight months. (See fig. 2 *B*.) This animal was also infected with S. pallida.

Moon,[6] discussing experimental cirrhosis of the liver, cited the objections of a number of authors to the use of rabbits in the study of this disease, because these animals are prone to coccidiosis and perhaps to

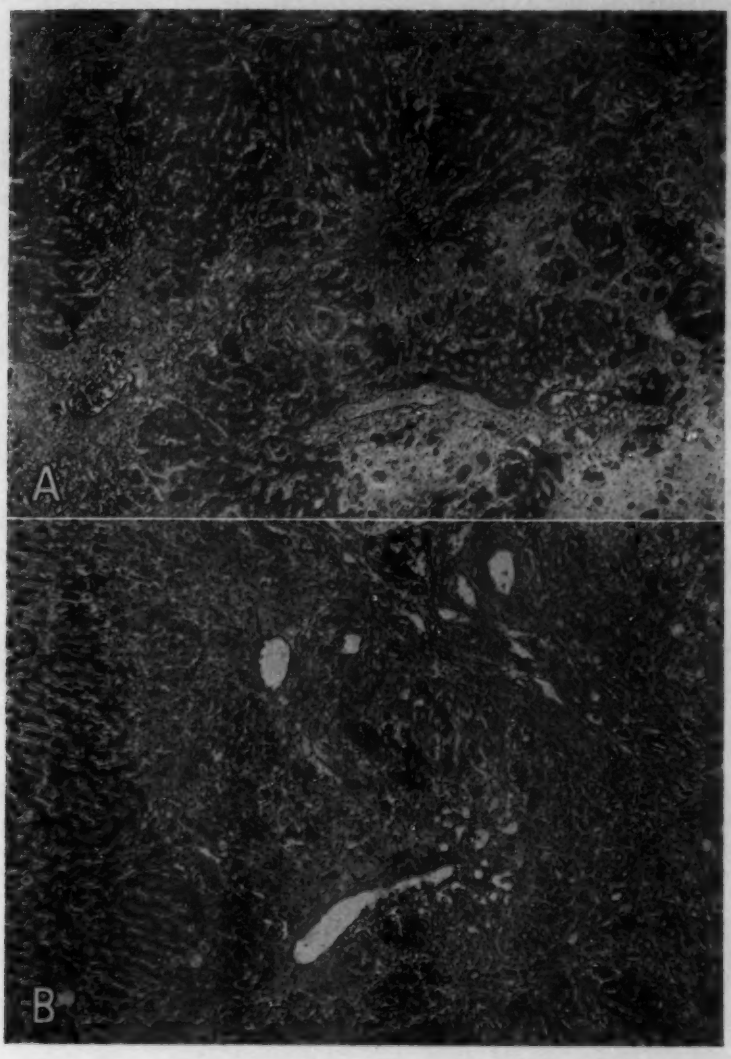

Fig. 2.—*A*, increase of connective tissue in the centers of the lobules with relatively little in the periportal areas. This rabbit (no. 29) had four subcutaneous injections of chloroform during one and a half months. Mallory's aniline blue stain; × 69. *B* (rabbit 11) extensive increase of fibrous connective tissue in the centers of the lobules with somewhat less in the peripheral parts. There is an increase of bile ducts. (See fig. 1 *B*.) Hematoxylin and eosin stain; × 69.

other infections which produce hepatic inflammation and even cirrhosis. These lesions, arising from the bile ducts, affect primarily the periportal tissue. Lesions such as those produced by chloroform, involving first the centers of the lobules, are not subject to this objection

SUMMARY

In rabbits repeated subcutaneous injections of chloroform produced at first central cirrhosis of the lobules of the liver. In the most advanced lesions produced, this proliferation of connective tissue and regeneration of liver cells resulted in a nodular cirrhosis similar in appearance to Laënnec's cirrhosis in man. Concomitant infection with S. pallida produced no significant increase in the incidence of these lesions.

HYPERPARATHYROIDISM SECONDARY TO EXPERIMENTAL RENAL INSUFFICIENCY

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AND
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Hyperparathyroidism secondary to renal insufficiency has been demonstrated in recent years by many authors.[1] Pappenheimer and his co-workers reproduced it in rats.[2] More recently we [3] showed that in human beings the increase in size of the parathyroid glands in renal insufficiency is accompanied by an increase in function.

The present theory as to the mechanism of the production of parathyroid hyperfunction under these circumstances is briefly as follows: The poorly functioning kidneys cause retention of inorganic phosphorus as well as of nonprotein nitrogenous compounds. The retained phosphate tends to depress the calcium and would indeed depress the calcium were it not for increased function and consequent hyperplasia of the parathyroid glands. The role played by the phosphate ion in this hypothetic chain of events was nicely demonstrated by Drake, Albright and Castleman,[4] who injected large quantities of phosphates intra-

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From the Department of Pediatrics, University of Chicago.

1. (*a*) MacCallum, W. G.: Bull. Johns Hopkins Hosp. **16**:87, 1905. (*b*) Bergstrand, H.: Acta med. Scandinav. **54**:539, 1921. (*c*) Hubbard, R. S., and Wentworth, J. A.: Proc. Soc. Exper. Biol. & Med. **18**:307, 1921. (*d*) Vines, H. W. C.: The Parathyroid Glands in Relation to Disease, New York, Longmans, Green & Company, 1924, p. 20. (*e*) Langmead, F. S., and Orr, J. W.: Arch. Dis. Childhood **8**:265, 1933. (*f*) Smyth, F. S., and Goldman, L.: Am. J. Dis. Child. **48**:596, 1934. (*g*) Shelling, D. H., and Remsen, D.: Bull. Johns Hopkins Hosp. **48**:596, 1935. (*h*) Cabot Case 22072, New England J. Med. **214**: 320, 1936. (*i*) Magnus, H. A., and Scott, R. B.: J. Path. & Bact. **42**:665, 1936. (*j*) Nelson, A. A.: Arch. Path. **24**:30, 1937. (*k*) Price, N. L., and Davie, T. B.: Brit. J. Surg. **24**:548, 1937. (*l*) Pappenheimer, A. M., and Wilens, S. L.: Am. J. Path. **11**:73, 1935. (*m*) Castleman, B., and Mallory, T. B.: ibid. **13**:553, 1937. (*n*) Gilmour, J. R., and Martin, W. J.: J. Path. & Bact. **44**:431, 1937.

2. Jarrett, W. A.; Peters, H. L., and Pappenheimer, A. M.: Proc. Soc. Exper. Biol. & Med. **32**:1211, 1935. Pappenheimer, A. M.: J. Exper. Med. **54**: 965, 1936.

3. Highman, W. J., Jr., and Hamilton, B.: J. Clin. Investigation **16**:103, 1937.

4. Drake, T. G.; Albright, F., and Castleman, B.: J. Clin. Investigation **16**: 203, 1937.

muscularly or intravenously or in both manners into rabbits over varying periods of time. They found that hyperplasia of the parathyroid glands was produced early and was a constant change in the animals so treated.

In the present work we have endeavored to ascertain the time at which hyperfunction of the parathyroid glands appears in dogs with experimental renal insufficiency. We have tried to determine whether there is an interrelationship between the amount of hyperplasia of the parathyroid glands and the amount of hyperfunction detectable by laboratory means. We have attempted to correlate changes in the serum calcium, serum phosphorus and blood nonprotein nitrogen with changes in parathyroid function.

METHODS

Experimental renal insufficiency in dogs was produced by an approximate heminephrectomy at one operation and a contralateral nephrectomy at a later operation. Tests for hyperfunction of the parathyroids were made on the blood of the dogs before operation and at intervals of varying numbers of weeks thereafter. Calcium, phosphorus and nonprotein nitrogen were determined on samples of blood drawn from the dogs at the same time as that for the parathyroid hyperfunction tests. The dogs were fed a normal diet of milk, bread and meat, and each was given a tablespoon of cod liver oil daily.

Tissues for routine microscopic inspection were fixed in Zenker's solution, with dilute solution of formaldehyde substituted for acetic acid, and embedded in pyroxylin. They were stained with hematoxylin and eosin. Fat stains were made on tissues fixed in solution of formaldehyde U. S. P. and stained with scarlet red. Blocks of bone were decalcified in formic acid and sodium citrate. Serum calcium was determined by the method of Fiske and Logan [5]; serum inorganic phosphorus, by the method of Fiske and Subbarow [6]; nonprotein nitrogen, by the method of Folin and Dennis.[7] The method of Hamilton and Schwartz [8] as modified by Hamilton and Highman [9] was used in performing the tests for hyperfunction of the parathyroids. This method consists in measuring the rise from the initial to the higher of the three or the five hour level of serum calcium in a fasting rabbit into which 30 cc. of the blood of the test animal or patient has been injected intramuscularly and to which calcium chloride has been administered by stomach tube at regular intervals following the injection of the blood. A rise of 0.30 millimol of calcium per liter of rabbit's serum is considered evidence of increased parathyroid activity in the animal or patient whose blood was injected into the rabbit.

For each parathyroid hyperfunction test on a particular dog the same rabbit was used as long as it survived so that the individual variation in the response of different rabbits to the injection of a fluid containing parathyroid hormone might be minimized.

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5. Fiske, C. H., and Logan, M. A.: J. Biol. Chem. **93**:211, 1931.
6. Fiske, C. H., and Subbarow, Y.: J. Biol. Chem. **66**:375, 1925.
7. Folin, O., and Dennis, W.: J. Biol. Chem. **23**:491, 1916.
8. Hamilton, B., and Schwartz, C.: J. Pharmacol. & Exper. Therap. **46**: 285, 1932.
9. Hamilton, B., and Highman, W. J., Jr.: J. Clin. Investigation **15**:99, 1936.

RESULTS

In figure 1 the values obtained from the blood of dog 1 are shown graphically. Before operation the parathyroid hyperfunction test gave a normal value. The values for calcium, phosphorus and nonprotein nitrogen in the blood were likewise normal. Three days after the second operation, when only about one fourth of the dog's original kidney tissue remained, the nonprotein nitrogen had risen to 272 mg. per hundred cubic centimeters of blood, the phosphorus had risen to 9.5 mg., the calcium remained normal, and the parathyroid function had risen, although it was still within the normal range. Throughout the subse-

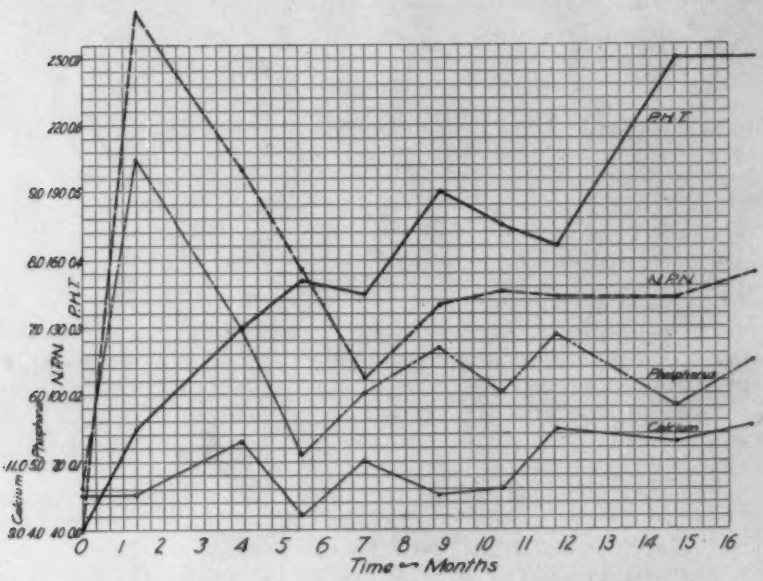

Fig. 1.—Correlation of chemical findings in the blood of dog 1 with values obtained by the parathyroid hyperfunction test (*P.H.T.*). *N.P.N.* signifies nonprotein nitrogen.

quent months the nonprotein nitrogen and phosphorus fell and then rose, the calcium remained within normal limits, while the tests for hyperfunction of the parathyroids showed a gradual but consistent increase. Figures 2 and 3 show the results obtained on the blood of dogs 4 and 3, respectively. Although neither of these dogs showed the degree of hyperazotemia which was found in dog 1, nevertheless in both the level of the nonprotein nitrogen in the blood was elevated. The serum calcium of both dogs remained within normal limits. Both showed an early rise in parathyroid function, which was maintained throughout the rest of their lives. The early high surge shown by the

parathyroid hyperfunction test on the blood of dog 4 is unexplained. The serum phosphorus level of dog 4 was elevated only slightly, and that of dog 3 was not elevated above normal limits.

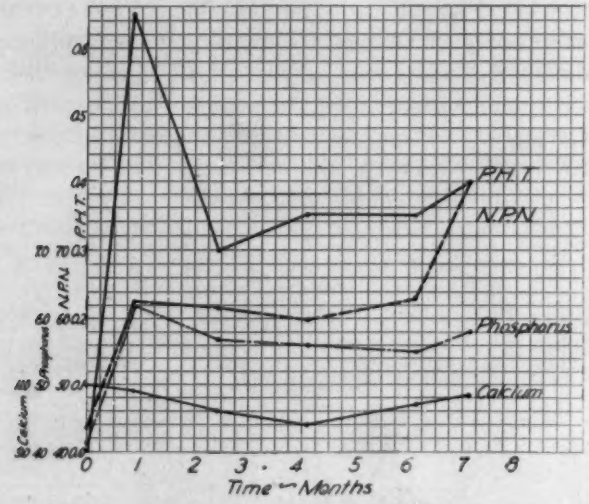

Fig. 2.—Correlation of chemical findings in the blood of dog 4 with values obtained by the parathyroid hyperfunction test (*P.H.T.*). *N.P.N.* signifies non-protein nitrogen.

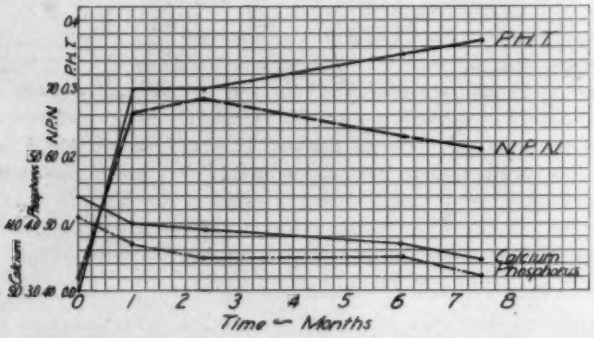

Fig. 3.—Correlation of the chemical findings in the blood of dog 3 with values obtained by the parathyroid hyperfunction test (*P.H.T.*). *N.P.N.* signifies non-protein nitrogen.

Other dogs died so soon after the second operative procedure that their detailed records do not seem worthy of inclusion among these data. However, the parathyroid glands of all of these dogs were grossly enlarged (fig. 4) so that in each case all four glands were clearly visible at autopsy. The enlargement of the glands of the dogs that

died early was roughly proportional to their time of survival. Microscopically, the glands showed hyperplasia, which was evaluated by increased packing of the cells, with destruction of the normal pseudoacinar pattern. The parathyroids of dog 1 showed complete obliteration of the glandular pattern by closely packed, deeply staining chief cells and early fibrosis. Those of the other dogs showed milder hyperplasia, commensurate with the degree of rise shown by the parathyroid hyperfunction test and with the duration and severity of their renal insufficiency.

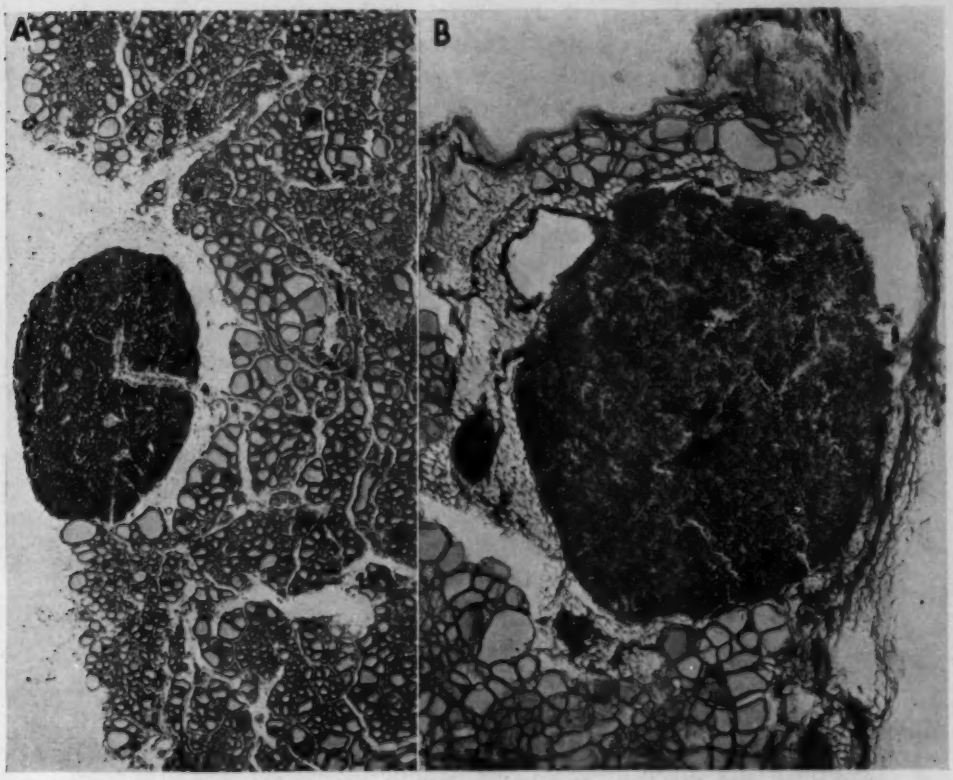

Fig. 4.—Parathyroid of a normal dog (*A*) compared with that of an experimental dog (*B*). The sections are cut at 8 microns through the plane of greatest area. There is great increase in size and marked hyperplasia in the parathyroid of the experimental dog, no. 9. This animal lived only seven weeks after operation. The section was selected to show how early marked parathyroid changes become evident. Magnification, × 23.

The residual portions of kidney showed an increase in fibrous tissue and an infiltration of round cells, with focal scars radiating into the renal parenchyma. There was abundant fibrous stroma, which contained

sparse atrophic tubules and shrunken glomeruli. Elsewhere the glomeruli were large and cellular and showed an increase in hyalin content suggestive of thickening of the basement membrane. In general the tubules appeared wide, containing much protein precipitate; also scattered throughout the cortex were cystlike tubules lined by low epithelium and containing hyaline or finely granular casts. Occasional widely disseminated foci of polymorphonuclear leukocytes were seen throughout the parenchyma. A moderate amount of fatty degeneration was present in the cortical epithelium.

Careful examination of the bones grossly, microscopically and in some instances roentgenographically revealed no changes suggestive of osteitis fibrosa. The cortices were normally dense. The trabeculae were well patterned. There was no fibrosis of marrow, osteoid proliferation or increase in osteoclasts or osteoblasts.

The heart, liver and lungs of every dog were examined as a routine and were not unusual. The spleen of each animal, however, and particularly that of dog 1, contained marked deposits of hemosiderin.

The thyroid of dog 1 showed several encapsulated calcific foci and many small cysts containing bluish granular, presumably calcified material. These cysts lacked epithelium, and where they were numerous there was abundant fibrous tissue, which was hyalinized and which incorporated small masses of calcium. A section of the aorta of dog 9 showed a rough, irregular lining with adherent thrombus material, granular deposits and many infiltrating cells. The calcium seemed to be concentrated in the internal elastic lamina. No other evidence of abnormal calcification was found in any of the animals.

COMMENT

In the 3 dogs which survived the experiment it is noteworthy that the serum phosphorus did not rise consistently inasmuch as these dogs showed, respectively, a markedly elevated, a moderately elevated and a normal level. The parathyroid function was increased in each instance, and the parathyroid glands were enlarged. In a previous paper [3] we presented data which indicated that parathyroid hyperfunction is more commonly found in hyperphosphatemic nephritic patients than in those whose phosphorus is at a normal level. Nevertheless, the instances in which parathyroid hyperfunction occurred without hyperphosphatemia are of sufficiently large percentage both in man and in dogs to indicate that the mechanism of parathyroid hyperfunction is as yet not fully understood.

In dogs as in human beings, there was observed no correlation between the rise of parathyroid function and the elevation of nonprotein nitrogen in the blood. But the most severe parathyroid changes did

take place in the dog whose nonprotein nitrogen reached the highest level, perhaps because he also lived the longest after the operative procedures. Castleman and Mallory [1m] presented an excellent discussion of the histologic picture of the parathyroids of human beings with chronic renal insufficiency. In this paper are tabulated among other findings the nonprotein nitrogen levels of the patients and the dimensions of their parathyroid glands, all of which showed chief cell hyperplasia. There was, however, no correlation between the elevation of the nonprotein nitrogen and the increase in size or weight of the parathyroid glands. Pappenheimer and Wilens [11] stated that the degree of parathyroid enlargement seems to be related to the severity of renal impairment. However, in their series of cases, no comparison was made between the extent of the parathyroid changes and the duration of the renal symptoms, nor were we able to make the latter comparison in our series of patients. It is conceivable that even though parathyroid changes, functional and morphologic, appear early, the changes are progressive and that the longer they have been taking place the more extensive they are. The results of our experiments on dogs seem to bear out this conception from the standpoint of parathyroid function. To justify the conclusion that this applies equally to the morphologic changes, many more animals must be used.

SUMMARY

Increased activity of the parathyroid glands as measured by the parathyroid hyperfunction test is detectable within the first month of experimental renal insufficiency in dogs. Hyperplasia appears at approximately the same time.

CASE OF PRIMARY RETICULUM CELL SARCOMA OF THE BRAIN

RELATIONSHIP OF MICROGLIA CELLS TO HISTIOCYTES

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The case to be presented is that of an unusual tumor of the brain, unique in the records of this hospital. It is definitely identifiable with only two tumors found described in the literature, to which the purely descriptive name "perithelial sarcoma" has been given.[1] Its evident mesodermal origin suggests a possible relationship to the microglia, so that a detailed study of the case has been made. This has been done in an effort to evaluate the hypothesis that a tumor of microglial origin would display the morphologic features of the tumors which arise from histiocytic elements elsewhere in the body.

REPORT OF CASE

An Italian aged 50, for a period of two weeks prior to admission to the hospital, had complained of drowsiness and severe headaches and had vomited on several occasions. Some weakness of the left arm and leg had been present for three or four days. He was known to have been diabetic for a questionable length of time. He was a well developed, fairly well nourished white man, extremely drowsy and weak. The pupils reacted to light. The tongue deviated slightly to the left, and the mouth deviated to the right on showing the teeth. The blood pressure was 120 systolic and 78 diastolic. There were noted some weakness of the left arm and leg without wasting, moderate hyperactivity of the tendon reflexes and a Babinski sign on the left side.

The urine had a specific gravity of 1.035. In all specimens sugar and acetone were 2 plus. The Hinton test was negative. The blood sugar varied from 364 to 285 mg. per hundred cubic centimeters during fasting. Lumbar puncture showed an immediate pressure of 240 mm. and a pressure of 140 mm. of mercury after withdrawal of 10 cc. of clear, pale yellow fluid. The cell count was lymphocytes 7 and red blood cells 9 per cubic millimeter. The Pandy test showed globulin 2 plus. The total protein was 120 mg. per hundred cubic centimeters. The Wassermann test was negative. The Lange colloidal gold curve was 011222110.

The patient remained in the hospital twelve days. The diabetes was uncontrollable, and the patient refused food by mouth after one week. Eight hours before death he became deeply comatose and died with rapid respirations, a temperature of 104 F. and markedly increased amounts of sugar in the urine despite treatment for diabetic coma.

From The Mallory Institute of Pathology, Boston City Hospital.

1. Bailey, P.: Arch. Surg. **18**:1359, 1929.

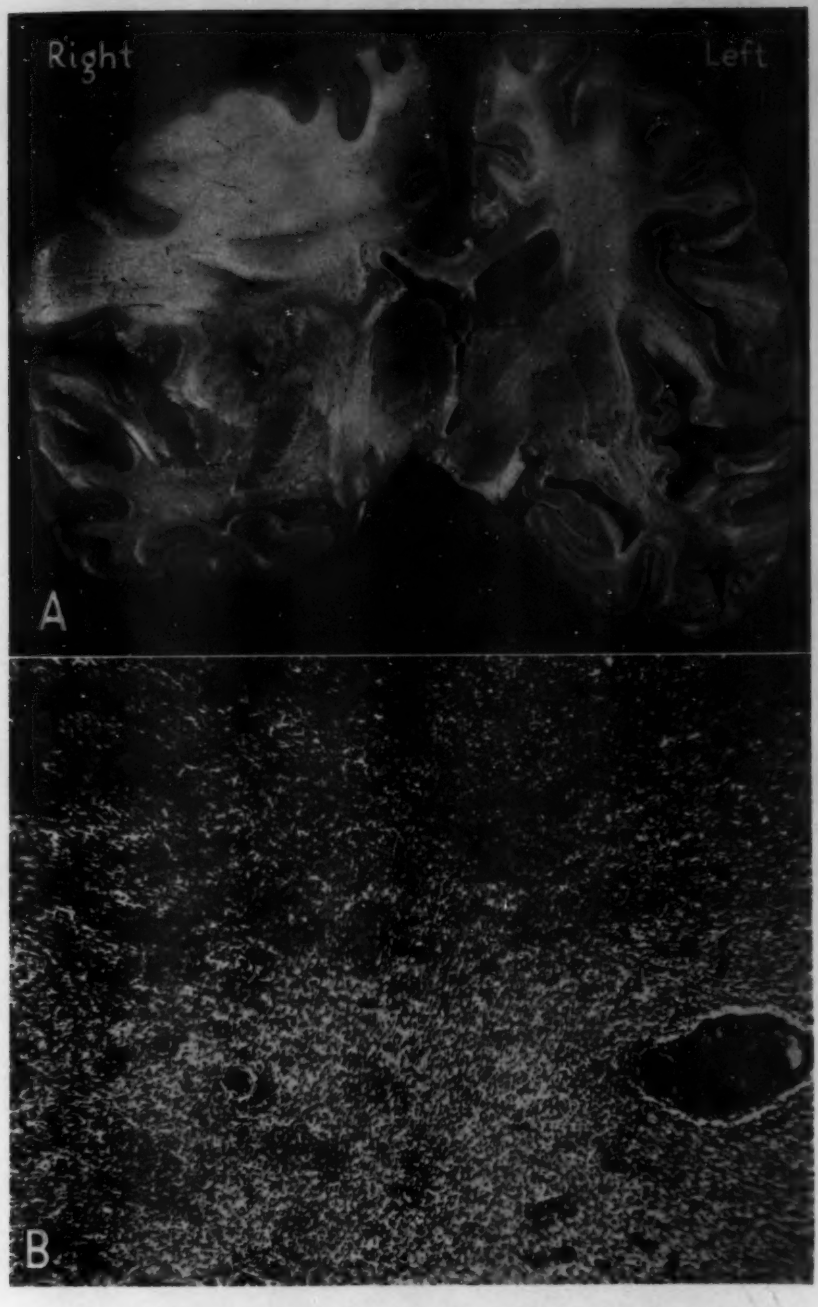

Fig. 1.—*A*, frontal section of the brain through the midportion of the tumor. *B*, low power photomicrograph showing the margin of the tumor; phloxine–methylene blue; × 37. Note the pseudoperivascular arrangement and fibrin deposits.

EXPLANATION OF FIGURE 2

A, high power photomicrograph showing the tumor cells around a small blood vessel; phosphotungstic acid-hematoxylin; × 500. The irregular cytoplasmic outlines can be seen. There are several phagocytic cells in the section. A multinucleated cell is marked (*g*) and a mitotic figure (*m*).

B, section of tumor prepared with reticulum stain; × 500. Note the concentric arrangement of reticulum around the blood vessel bearing no relationship to the surrounding tumor cells.

C, section of tumor prepared with Hortega's silver-carbonate stain; × 500. The large cell shows long branching processes. Several resemble certain transitional forms of the microglia cells, and others have short irregular impregnated processes.

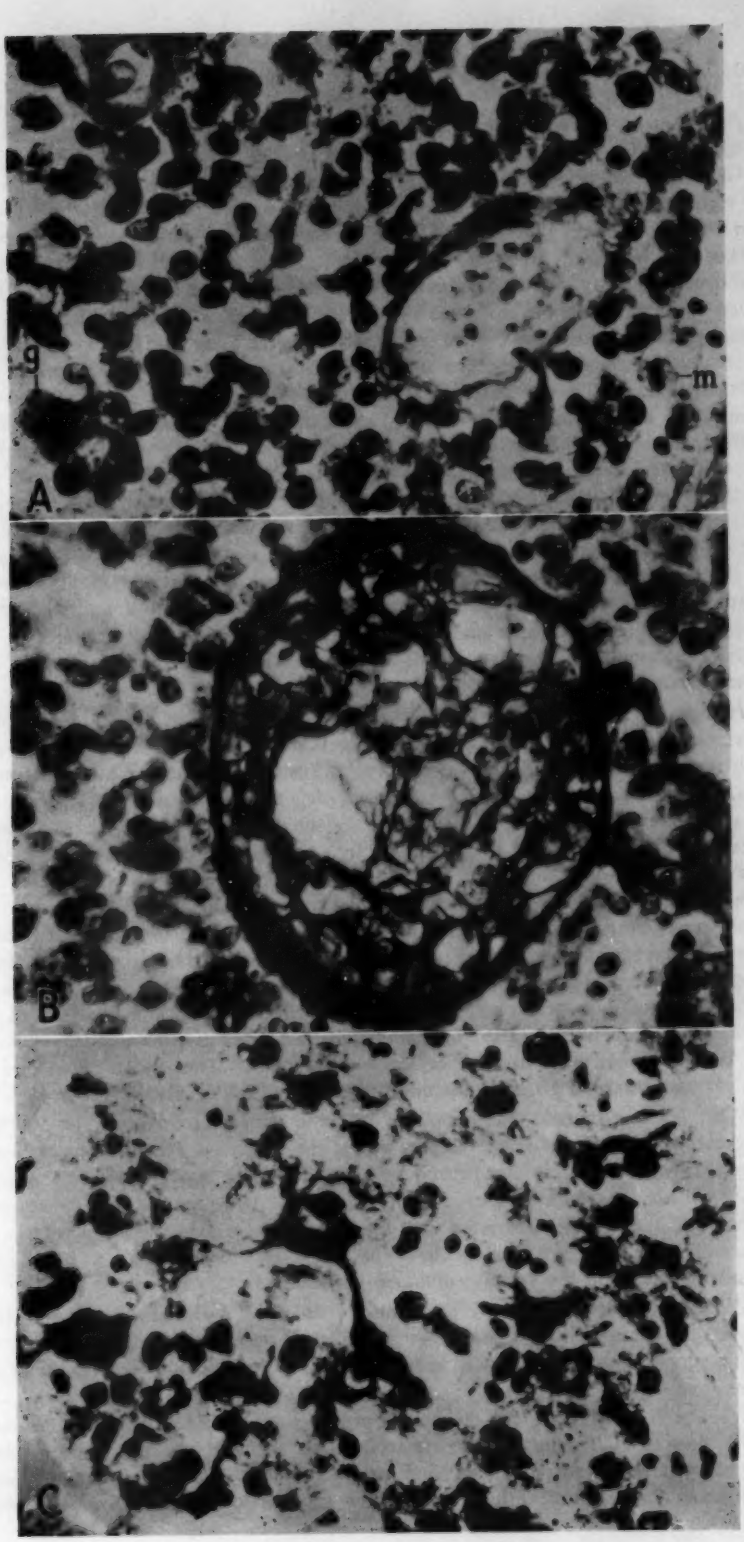

Figure 2

Postmortem Examination of the Brain.—The brain (fig. 1 *A*) revealed an extreme degree of cerebral edema, most marked on the right, and a grayish pink tumor mass, measuring 2.8 by 2.5 by 2.5 cm., situated in the central portion of the right hemisphere. It was not sharply circumscribed but was slightly firmer than the surrounding brain tissue. The cerebral edema, which was much more marked on the right, involved only the central and convolutional white matter. The overlying gray ribbon was unaltered in some places; in others it was narrowed by compression. The anterior limit of the tumor was at the level of a frontal section through the central sulcus, crossing the body of the caudate nucleus, the massa intermedia of the thalamus and the midportion of the putamen and globus pallidus. The posterior limit was at the level of the pulvinar and the splenium of the corpus callosum. The lateral margin compressed the gray matter of the insula. The medial border pushed the internal capsule toward the midline and dislocated it into a perpendicular position. The thalamus was distorted and displaced somewhat across the midline; the right lateral ventricle was distorted and narrowed, and the putamen and globus pallidus were displaced inferiorly and slightly toward the midline. None of these structures seemed to be invaded by the tumor. However, the following parts had been destroyed: claustrum, external capsule, association pathways passing through the insula, and the lateral edge of the internal capsule on the right. Examination of the remainder of the body gave essentially negative results except for bilateral bronchopneumonia and acute and chronic seminovesiculitis.

Histologic Examination.—With phloxine–methylene blue (tetrabromdichlorfluorescein) (fig. 1 *B*) a large area of the section of brain, indistinctly demarcated from the relatively normal brain tissue, was diffusely infiltrated with large atypical cells. These had irregular round to oval nuclei, ranging in diameter from about 6 to 10 microns, many of which were indented. Each nucleus had a heavy chromatin network without prominent nucleoli. There were many mitotic figures, as well as cells containing from 2 to 4 nuclei. There was a moderate amount of cytoplasm, irregular in outline and showing small pseudopodic projections. Many of the cells contained phagocytosed cellular material. The cells were closely packed in the perivascular spaces, spreading out from them into less cellular areas, in which there were heavy fibrin deposits, scattered large astrocytes and macrophages containing fat vacuoles and cellular débris. The walls of many of the blood vessels had been invaded by tumor cells. Around the margins the infiltration was more diffuse with less evidence of necrosis. The tumor faded into an area showing marked gliosis containing numerous fibrillary astrocytes.

With phosphotungstic acid–hematoxylin stain (fig. 2 *A*) no fibrils or vascular attachments were observed in relation to the tumor cells. The blunt cytoplasmic processes were more prominent, and many of the cells appeared comma shaped.

With reticulum stain (Hortega-Foot) (fig. 2 *B*) there was a well impregnated coarse reticulum closely associated with the tumor cells around the blood vessels. It apparently bore no direct relationship to the neoplastic cells, however, as many large groups farther from the vessels contained no reticulum.

With gold chloride (Cajal) the tumor cells were indistinct, staining a faint purple, and were without visible processes. Well stained astrocytes were present in the surrounding brain tissue, and a few were seen scattered throughout the tumor.

With silver carbonate (Hortega) for microglia (fig. 2 *C*) most of the tumor cells stained well, the nuclei varying from black to dark brown. The cytoplasm showed variation in shape, amount and staining properties. Some cells were round or comma shaped; others had short blunt or rounded pseudopodic projections,

and many had long unipolar and bipolar processes, varying greatly in thickness. These were usually branched and showed numerous small spinous excrescences. In some cells the cytoplasm appeared colorless, finely granular or honeycombed, with a thin black line around the margin; in others, particularly those with the most marked processes and those containing phagocytosed material, it was dark brown. Short, well stained processes were present in many of the multinucleated and dividing cells. Around the margin of the tumor were numerous mature and transitional forms of microglia. The astrocytes stained faintly and were without visible processes.

Differential Diagnosis.—The following tumors can be excluded on histologic grounds:

Glioblastoma multiforme. The type cell was too uniform in both size and shape.

Astrocytic group. The Cajal stain impregnated the incidental astrocytes but not the tumor cells.

Ependymoma. There was no evidence of cyst formation, cilia or lining-up of the tumor cells, and no blepharoplasts were seen in the phosphotungstic acid–hematoxylin stain.

Medulloblastoma. The age of the patient and the situation of the tumor tend to exclude this type. The nuclei did not have the usual regular ovoid shape, and there was no tendency to rosette formation.

Oligodendroglioma. The structure was not sufficiently uniform; the nuclei had a heavy instead of a light chromatin content, and the processes were not characteristic.

COMMENT

The histologic structure of the tumor appears to be identical with that in 2 cases of "perithelial sarcoma" described by Bailey in 1929.[1] About 10 other cases of so-called perithelioma involving the brain and meninges have been described in the literature. These cases bear only a superficial resemblance to the present case. No definite attempt was made to determine the exact origin of the tumor in any of these cases; it is merely stated that it arose from adventitial cells in the perivascular spaces. These adventitial cells have been conclusively shown by various investigators to be histiocytes similar to those found elsewhere in the body, in connective tissue and parenchymatous organs, whether perivascular or interstitial. In common with these, the adventitial cells in the brain phagocytose various dyes, colloidal substances, bacteria and products of the breakdown of tissues, being the only cells in the brain to perform all the functions of the so-called reticuloendothelial system under normal conditions.[2]

The reticulum cell sarcoma is believed to arise from the undifferentiated mesenchymal cell, the derivatives of which are the various tissue phagocytes of the body. This type of tumor is found most frequently in lymph nodes but also in the gastrointestinal tract, bones, spleen and other parenchymatous organs. It is a uniformly cellular

2. (*a*) Kubie, L. S.: J. Exper. Med. **46**:615, 1927. (*b*) Lebowich, R. J.: Arch. Path. **18**:50, 1934. (*c*) Bratiano, S., and Llombart, A.: Ann. d'anat. path. **6**:849, 1929.

tumor, composed of anaplastic cells with round to oval or kidney-shaped nuclei, which are indented. The nucleoli are not prominent. There is a relatively large amount of cytoplasm, which shows signs of active motility, with irregular pseudopodic projections. Variable numbers of multinucleated cells, containing from 2 to 4 nuclei, are found. Reticulum cell sarcoma occurs in the older age group.

It follows logically that a similar tumor could arise from the adventitial histiocytes in the brain. The case under consideration fulfils all the morphologic criteria of a reticulum cell sarcoma. However, there is no definite evidence of its perivascular origin. Unquestionably there were many perivascular spaces filled with tumor cells, but in other areas the infiltration of the brain substance was diffuse, and the deposition of fibrin and marked phagocytosis occurring at a distance from the blood vessels suggested a pseudoperivascular effect. The tumor might readily have arisen elsewhere in the brain and been merely growing along the perivascular spaces. The only other mesenchymal element in the brain from which such a tumor could conceivably have arisen was the microglia cell.

Nowhere in the literature has a tumor of microglial origin been described. However, Bailey and Cushing [3] considered the possibility in a case of theirs. In reviewing it, they stated:

> It may be recalled in favor of this view: (1) that the cells were impregnated sharply by the microglial technique, (2) that they were not impregnated by methods specific for the neuroglial cells; (3) that the tumor is one of very unusual structure, unique in this series; and (4) that the nuclei closely resemble those of microglial cells. Against this view might be urged: (1) that the processes do not show the lateral excrescences characteristic of microglial cells; (2) that the processes of some of the cells are attached to the vessel walls in a manner similar to "sucker feet"; and (3) that the tumor is but an isolated example with no confirmatory evidence.

They concluded:

> Since the microglial cells have very definite structural characteristics, it seems to us not permissible to state that a tumor is derived from them unless its cells show the peculiarities possessed by the normal microglia cells.

The possible relationship of the tumor under consideration to the microglia was suggested not only by such strictly morphologic criteria as those of Bailey and Cushing but also by the theoretic concept that the microglia could give rise to a tumor resembling a reticulum cell sarcoma. Different workers using various methods of approach have recently done much work on the histogenesis of the microglia and its relationship to histiocytes and the so-called reticuloendothelial system. A brief review of the literature will be made in an endeavor to find a basis for this assumption.

3. Bailey, P., and Cushing, H.: A Classification of Tumors of the Glioma Group, Philadelphia, J. B. Lippincott Company, 1926.

Hortega [4] concluded that the microglia cells arise late in embryonic life from mesenchymal cells in the pia mater and also from the pia of large and medium-sized blood vessels, these regions being particularly rich in histiocytes. They migrate to all parts of the central nervous system as early or transitional forms, varying in shape from large round cells through irregular forms with blunt pseudopodic processes to elongated rods. The nuclei are round to slightly angular, with abundant chromatin. The fully developed resting forms, found in normal brain tissue, show variations in the number, thickness and branching of their processes, depending on their location and the density of the surrounding structures. In response to inflammatory and necrotizing processes the microglia repeats its early developmental stages, ultimately forming macrophages (fat granule cells). This reaction is essentially different from the degenerative changes seen in nerve and glial cells under similar circumstances. It is generally conceded that morphologically identical fat granule cells also arise from the adventitial histiocytes around the blood vessels.

Physiologically, the microglia cell is assumed to represent the specialized reticuloendothelial element in the central nervous system. The hemolytic and phagocytic functions of the microglia cell are undisputed. However, under normal conditions the fixation of colloidal substances has been shown to occur only in the histiocytes of the pia, choroid plexus and adventitia of the blood vessels. This has been demonstrated by both intravital and supravital methods.[5] Various workers have succeeded, after injury to the brain, either traumatic or inflammatory, in staining transitional forms of the microglia cell and fat granule cell with such dyes as trypan blue, pyrrole blue and lithium carmine, injected intravitally. No fixation of colloids has ever been noted in the resting mature forms. Bratiano and Llombart,[2c] using lithium carmine, concluded that the effect obtained after injury is not true colloidopexie, the dye being found in the adventitial histiocytes as discrete granules, whereas in the microgliocytes it is diffuse. They question whether this is due to phagocytosis or to cell injury and exclude the microglia from the reticuloendothelial system on this basis.

By means of the silver carbonate method, cells have been demonstrated in the heart, striated muscle and bladder by Visintini [6] and in the liver, spleen, kidney and peritoneal membrane by Dunning and Furth [7] which are morphologically identical with the microglia cells.

4. del Rio Hortega, in Penfield, W.: Cytology and Cellular Pathology of the Nervous System, New York, Paul B. Hoeber, Inc., 1932.

5. Russell, D. S.: Am. J. Path. **5**:451, 1929. Zand, N.: Ann. d'anat. path. **7**:565, 1930. Bailey and Cushing.[3]

6. Visintini, F.: Rev. neurol. **1**:906, 1930.

7. Dunning, H. S., and Furth, J.: Am. J. Path. **11**:895, 1935.

Dunning and Stevenson [8] have also shown that the reaction of these cells to injury and to intravital injection of trypan blue is exactly similar to that of microglia cells.

Costero in 1930 demonstrated in tissue cultures of brain explants large ameboid cells with all the morphologic and functional characteristics of the microglia cells. He obtained these in pure culture and claimed that they were microgliocytes. Dunning and Stevenson,[8] however, have grown similar cells with identical properties in cultures of brains and livers of chicken and guinea pig embryos and in cultures of the kidney of a chicken embryo.

In view of the more recent experimental data, the only remaining bases for considering the microglia cells as a definite entity, distinct from the histiocytes elsewhere in the body, seem to be slight variations in the shape and functions of the mature resting forms. Although the final links of proof are missing, it seems reasonable to assume that these variations are secondary to the highly specialized structure of the brain itself.

Before attempting to draw any conclusions concerning the present tumor, the question of the derivation of the numerous macrophages found in it remains to be considered. The following points favor the view that they were actively phagocytic tumor cells: 1. The tumor cells and the macrophages had similar nuclei, and the cytoplasm of both was sharply stained by the silver carbonate method. 2. The cytoplasmic outlines of the least differentiated tumor cells suggested active ameboid motility. 3. Many of the tumor cells were morphologically identical with the various transitional forms of the microglia cells and tissue histiocytes seen during their development into macrophages, both in fixed sections and tissue cultures. 4. Such large numbers of macrophages are not usually seen in a tumor of the brain, and the degree of necrosis present did not appear to be sufficient to have called forth such a marked response from without. 5. Phagocytosis may be present in any type of tumor cell and would be expected in a tumor arising from the same stem as the macrophage.

SUMMARY

A case of primary tumor of the brain of mesenchymal origin has been presented, which was identified morphologically as a reticulum cell sarcoma. In sections stained with silver carbonate the tumor cells displayed the histologic characteristics of transitional forms of microglia cells. It is believed that this case presents further evidence of the histiocytic nature of the microglia.

8. Dunning, H. S., and Stevenson, L.: Am. J. Path. **10**:343, 1934.

ERYTHROBLASTS IN THE CHORIONIC BLOOD VESSELS OF HUMAN EMBRYOS

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AND
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Erythroblasts predominate in the blood vessels of the chorionic villi of the human embryo, rapidly disappear toward the end of the third month of pregnancy and are practically absent in the chorionic blood vessels of the fetus. The practical and theoretic importance of these observations made by Ryerson and Sanes [1] and confirmed by Ohringer [2] prompted their further investigation.

MATERIAL AND METHODS

From routine microscopic preparations of uterine scrapings containing placental tissue (material fixed in solution of formaldehyde U. S. P. diluted 10 per cent, embedded in paraffin, cut and stained with hematoxylin and eosin), 38 specimens were chosen in which the blood vessels of the chorionic villi contained nucleated red blood cells. To these were added 30 surgically removed pregnant fallopian tubes and 12 embryos with cords, placentas and membranes. The proportions of nucleated and non-nucleated red blood cells in the chorionic blood vessels were determined in each instance. Then, all information relevant to the duration of pregnancy, such as the date of the onset of the last menstrual period, the type of menstrual cycle and its usual duration, the date of operation and the length of the embryo, was tabulated. From these data, the maximal duration of pregnancy was estimated in each case by subtracting from the number of days elapsed between the onset of the last menstrual period and the operation the number of days plus one of the usual duration of menstruation. When the information was stated in general terms such as "missed one period" and the type of cycle was not recorded, the maximal duration of pregnancy was estimated on the basis of a twenty-eight day cycle with five days' duration. A variation of about ten days in either direction naturally must be allowed in all instances.

OBSERVATIONS

In the first group, 38 cases in which the material was obtained by curettage, the chorionic blood vessels contained less than 100 per cent erythroblasts in 8 instances. The estimated maximal duration of pregnancy in these varied between forty-five and eighty-four days, with an

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1. Ryerson, C. S., and Sanes, S.: Arch. Path. **17**:648, 1934.
2. Ohringer, L.: Centralbl. f. allg. Path. u. path. Anat. **63**:373, 1935.

average of sixty-five days. In all the others, 30 cases, the erythroblast content of the chorionic blood vessels was 100 per cent. Among these, the maximal duration of pregnancy varied between twenty-seven and seventy-nine days, with an average of fifty-one days.

In the second group, 30 cases of tubal pregnancy, in 1 case the erythroblast content of the chorionic blood vessels was 3 per cent and the length of the fetus was 100 mm. In all the other 29 instances the erythroblast content of the chorionic blood vessels was 100 per cent. The maximal duration of pregnancy, known in 18 of these cases, varied between twenty-six and sixty-one days, with an average of forty-four days. The size of the embryos, known in 10 instances, varied between 13 and 30 mm.

In the third group, 12 embryos with cords, placentas and membranes, the erythroblast content of the chorionic blood vessels was 100 per cent in all but a single case. The length of the embryos, known in 10 instances, varied between 10 and 45 mm. The maximal duration of pregnancy varied between thirty-four and seventy-nine days with an average of fifty days.

From the three groups studied, it is evident that erythrocytes first appear in the chorionic blood vessels near the beginning of the third month of pregnancy and increase to over 90 per cent by the end of the third month.

SUMMARY

The erythroblast content of the chorionic blood vessels was investigated in placental tissue in 80 specimens, including 30 pregnant fallopian tubes and 12 embryos. In the 70 cases in which the chorionic blood vessels contained only nucleated red blood cells the estimated maximal duration of pregnancy was two months or less. In the remaining cases in which the erythroblast content of the chorionic blood vessels was not less than 10 per cent the estimated maximal duration of pregnancy was between two and three months.

These observations, apart from their practical implications in determining the approximate duration of pregnancy, have considerable theoretic interest; namely, they open a new field for the study of the development of red blood cells, the factors leading to their maturation and those preventing their maturation, as in erythroblastosis foetalis.

METASTATIC CALCIFICATION IN A CASE OF CARCINOMA OF THE BREAST

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PHILADELPHIA

Virchow [1] described several cases of primary tumor and secondary tumor of bone with calcific deposits in the lungs, kidneys and stomach. For these deposits he coined the term "metastatic calcification," implying supersaturation of the blood with calcium salts previously absorbed from the bones of the body and deposition of these salts in tissues otherwise normal. This view received universal acceptance, as metastatic calcification was found to accompany other diseases associated with marked destruction of bone, i. e., osteomyelitis, tuberculosis, leukemia and hyperparathyroidism.

Wells [2] reviewed cases of calcification and noted as a striking feature that the sites of predilection for deposition of calcium were the chief avenues for the elimination of acid from the body, viz, the lungs, kidneys and stomach. He further noted the importance of the carbon dioxide tension of the blood and of the tissue fluids in the transportation of calcium salts to, and their deposition in, the pulmonary alveolar walls and veins and the left side of the heart.

Interesting in this connection is the experiment of Burge.[3] He injured the intima of the carotid artery and found it electronegative. He attributed this to negatively charged phosphate ions and the subsequent calcification to combination with the positive calcium ions of the blood, thus precipitating insoluble calcium phosphate.

Recent experimental work [4] showed that degeneration of the renal parenchyma occurs constantly and early in calcinosis and that it probably precedes the deposition of calcium. With the phosphate radicles and the albuminous substances in the blood keeping calcium salts in solution, their depletion during nephritis favors precipitation of calcium. Instances of metastatic calcification with involvement of the endocardium are rare.

From the Department of Pathology, Rhode Island Hospital, Providence, R. I.

1. Virchow, R.: Virchows Arch. f. path. Anat. **8**:108, 1855.
2. Wells, H. G.: Arch. Int. Med. **7**:721, 1911; **15**:574, 1915.
3. Burge, W. E.: Arch. Path. **20**:690, 1935.
4. Reed, C. I., and others: J. Nutrition **6**:371, 1933.

REPORT OF CASE

A 30 year old white woman was admitted from the Providence Lying-In Hospital to the medical service of Dr. Charles F. Gormly, because of a history of increasing weakness and attacks of severe shooting pains in the shoulders, back and lumbar region, markedly aggravated by muscular effort, so that eventually she was forced to bed. Her complaints began in the sixth month of the fourth pregnancy and persisted after delivery of a macerated fetus, two months later. At this time roentgen studies revealed multiple punched-out areas in the calvarium, vertebrae, femurs and humeri. A diagnosis of multiple myeloma was entertained until a biopsy from a lesion of one of the ribs revealed adenocarcinoma, probably secondary to a tumor of the breast.

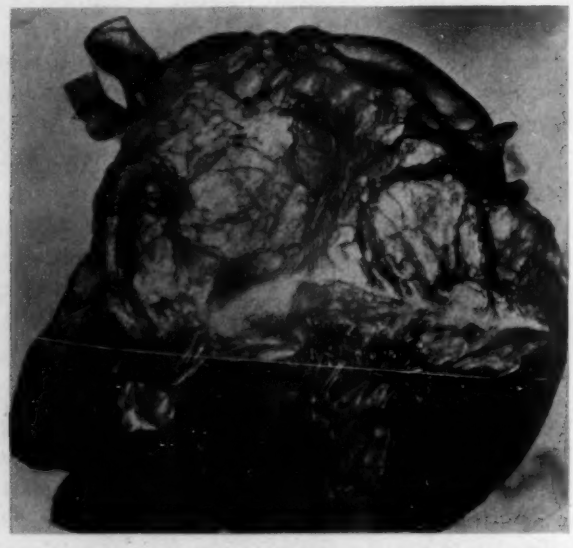

Fig. 1.—The left side of the heart with the eggshell-like deposits covering the entire left auricular endocardium with the exception of the fibrous sulcus running down its center to the intact mitral valve.

The blood, Feb. 10, 1936, showed: urea nitrogen, 39 mg. per hundred cubic centimeters; creatinine, 2.1 mg.; dextrose, 79 mg.; calcium, 18.2 mg.; ionized calcium, 9 mg.; phosphorus, 5 mg.; total protein, 5.8 Gm. The albumin-globulin ratio was 0.9, with albumin 2.6 and globulin 2.9 Gm. per hundred cubic centimeters.

Two days later the blood revealed: urea nitrogen, 42 mg.; creatinine, 2.1 mg.; dextrose, 72 mg.; calcium, 18.7 mg.; phosphorus, 4.1 mg.; total protein, 5.5 mg. Bence-Jones protein was absent from both blood and urine. Gastric analysis failed to reveal free hydrochloric acid.

The patient died February 16, three months after the onset of her symptoms. A necropsy was made one hour after death.

Observations at Necropsy.—The body was that of a pale, emaciated white woman. In the medial aspect of the right breast there was an irregular oval firm mass, 3 by 2 by 2.5 cm. in diameter, unattached to the skin and slightly fixed to the pectoral muscles. On section it was hard, firm and gray, with numerous

comedo-like areas of necrotic material scattered over its surface. No nodes were palpable in either axilla. The para-aortic nodes from the bifurcation of the trachea to the iliac arteries were enlarged and replaced by tumor. A few glands along the hepatoduodenal ligament were also involved. Scattered over the anterior surface of the left lobe of the liver were a few round flattened superficial nodules 0.5 cm. in diameter.

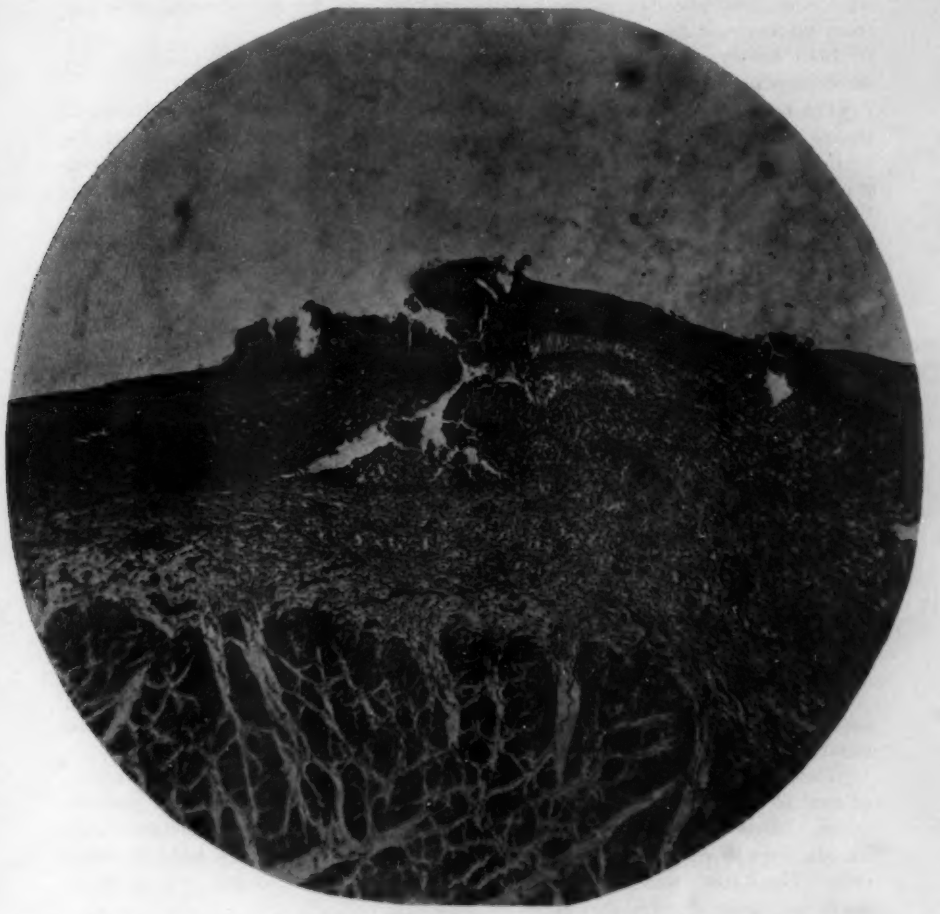

Fig. 2.—Left auricle showing the superficial thickened hyaline band beneath the endocardium. Below it are fragmented elastic fibers containing numerous calcium granules (staining black). In the center the calcified layer has ruptured through the endocardium to the surface. Hematoxylin and eosin; × 40.

The heart weighed 350 Gm., was of moderate firmness and showed a dilated left auricle. All the chambers contained "currant jelly" clots. The endocardium of the left auricle was covered with a brittle, fragmented eggshell-like material. These deposits encrusted with broken plaques the entire endoauricular surface excepting the appendage and a longitudinal sulcus extending from the ostia of

the pulmonary veins to the attachment of the mitral leaflets. The mitral cusps were thin, pliable and free from sclerotic changes or calcific deposits. Along the attachment of the aortic cusps there were a few small calcified plaques which resembled others discretely scattered over the intima of the aorta, regarded as simple atheromas. Serial sections along the coronary arteries and through the myocardium failed to reveal degenerative changes or further deposits of calcium.

Each lung had the consistency of a bath sponge. On section no tumor nodules were seen; the branches of the pulmonary veins and the parenchyma were free from macroscopic deposits of calcium.

Both kidneys were edematous and mottled gray. No grossly calcified areas were identified.

The neck failed to reveal enlargement or increase in number of the parathyroid bodies. Eight brownish gray, matchhead-sized structures were found in the region of the parathyroids. Three of these on microscopic review were seen to be parathyroid glands showing no evidence of hyperplasia. The remaining five were lymph nodes. The ribs and vertebrae showed the medullary cavities to be filled with pearly gray firm masses of tumor tissue. The ribs contained numerous nodular excrescences, varying from 0.5 to 2 cm. in diameter, most of which presented on the ventral segments.

The tissues were fixed in Zenker's solution, and those of the auricle and ribs were decalcified with formic acid. Since Ham [5] found a close correlation between the deposits stained by hematoxylin and those shown to be calcium by the incineration technic of Policard, my associates and I have interpreted material taking the hematoxylin stain as indicating calcific areas.

Microscopic Examination.—Sections of the left auricle (fig. 2) presented a flattened endocardial layer underlaid by a hyalinized zone diffusely impregnated with calcium, which stained purple-pink. Beneath this acellular layer lay swollen and fragmented elastic fibers containing numerous granules of varying size, surrounded by a few lymphocytes. The myocardial fibers were normal. In several areas the calcium had penetrated the hyaline layer and reached the surface.

Sections of the lung showed fibrosed and thickened alveolar septums containing calcium granules; numerous calcified plaques lay in the media of the pulmonary veins, and the submucosa of a bronchiole was similarly affected.

The kidneys contained calcium deposits in the glomerular tufts, and calcium casts were seen within dilated convoluted tubules, the lining epithelium being swollen, granular and frequently detached.

The tumor of the breast was composed of compact tubular and alveolar masses of oval to cuboidal epithelial cells with clear cytoplasm and large round vesicular nuclei. Mitotic figures averaged from 1 to 3 per high power field. Between these masses were dilated ducts and medium-sized arteries, their lumens lined by tumor cells. The stroma was dense and contained a scattering of plasma cells about the ducts and large discrete masses of lymphocytes.

The periaortic lymph nodes and the liver were the sites of metastatic tumors. The stomach was normal.

COMMENT

Calcific deposits indistinguishable from those just described have been produced experimentally. Tanaka in 1911, on injecting soluble calcium salts into the peritoneal cavities of animals, observed calcium

5. Ham, A. W.: Arch. Path. **14**:621, 1932.

deposited in the alveolar septums of the lungs, the epithelial cells of the bronchiolar mucosa and the convoluted tubules of the kidneys, apart from local deposits. Katase [6] obtained a similar result by injecting soluble calcium salts subcutaneously and intravenously. Learner,[7] from his experiments with parathyroid extract and a review of earlier work, interpreted the deposits as a result of renal damage. This view was further corroborated by Hueper,[8] who in the course of a routine histologic examination of the organs of 75 rats used for experimental purposes found calcific plaques in the branches of the pulmonary artery in 12, none of the other vessels or organs being involved.

Similar lesions were produced by injections of parathyroid extract (McJunkin;[9] Learner [7]), but in these cases there was always necrosis of the soft tissues of the heart and kidneys primarily, the deposition of calcium following. Shelling [10] demonstrated like effects following large doses of viosterol. These phenomena can hardly be included under the name "metastatic calcification" in its usual connotation.

Hypercalcemia is not necessarily the result of hyperplasia of the parathyroids, as Gutman [11] observed in a study of bone diseases; in cases of malignant growth such as that described here, it is due to rapid dissolution of bone.

SUMMARY

A case of adenocarcinoma of the breast is reported in which there occurred extensive metastasis to bones, hypercalcemia (without hyperparathyroid activity) and metastatic calcification. The sites of calcification were the alveolar septums of the lungs, the media of the pulmonary veins, the submucosa of the bronchioles and the left auricle of the heart. In the kidneys, calcific casts were present in the tubules, and calcium granules studded the glomerular tufts.

6. Katase, A.: Beitr. z. path. Anat. u. z. allg. Path. **57**:516, 1914.
7. Learner, A.: J. Lab. & Clin. Med. **14**:921, 1929.
8. Hueper, W. C.: Arch. Path. **20**:708, 1935.
9. McJunkin, F. A.: Arch. Path. **14**:649, 1932.
10. Shelling, D. H.: Proc. Soc. Exper. Biol. & Med. **28**:307, 1930.
11. Gutman, A. B.: Arch. Int. Med. **57**:411, 1936.

Case Reports

TULAREMIA IN AN INFANT WITH EXTENSIVE INTESTINAL LESIONS

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The case of tularemia to be reported is of unusual interest because of the character and distribution of the lesions, the lack of known exposure to the disease, and the age of the patient.

REPORT OF CASE

The patient was a white girl 12 months of age. Her development had been normal. She walked at 9 months and had been entirely breast fed. Later questioning produced no information that the child had been given anything to eat other than breast milk and on a few occasions "barley water." She had been kept indoors practically all the time but had been taken outside into the yard on one occasion a few days before the onset of illness. At that time, it was said, she had been carefully watched, and the mother felt certain she had not picked up anything to eat.

The family lived on a farm in southeastern Missouri. The father slaughtered cattle for the farmers in the surrounding territory. He had not been sick. Besides the patient, the family consisted of the father, mother, grandmother and four children between 5 and 11 years of age. A few days before the infant became ill, the mother, grandmother and two other children had a transient illness of one or two days' duration, characterized by vomiting and diarrhea. None of the farm animals were known to have been sick in recent weeks. There were, however, three large collie dogs which ran wild and occasionally brought in animals caught in the woods. A rabbit had been caught by one of the dogs and had been cooked and eaten by the family, but this occurred two months before the infant's illness.

Two weeks before death, the infant became ill with diarrhea, vomiting and fever. A few days later, one and one-half weeks before death, a nasal discharge and cough developed, and a local physician made a diagnosis of pneumonia in the upper lobe of the right lung. The child was admitted to the St. Louis Children's Hospital two weeks after the onset of illness. For the previous few days the extremities had stiffened at times as though in tetany. The diarrhea had continued from the onset of the illness, the stools varying in number from two to twelve per day and containing no blood or mucus.

On entry the patient was found to be extremely ill, showing irregular respirations with periods of apnea, and was cyanotic. There was mottling of the skin of the extremities. The ear drums showed injection and were full, and the pharynx showed marked injection. There was slight impairment of resonance, with suppression of breath sounds over the upper part of the right lung posteriorly. The heart rate was rapid, and the sounds were faint. There were periods of stiffening of the extremities lasting from two to three minutes. A tourniquet placed on the arm gave a definite carpopedal spasm. The abdomen

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was slightly distended; no tenderness or rigidity was noted. Fluoroscopic examination of the lungs showed a shadow in the periphery of the upper lobe of the right lung.

The child failed to respond to symptomatic treatment. Its temperature remained between 39 and 40 C. (102.02 and 104 F.) and it died a few hours after admission. A blood culture was made in which the influenza bacillus was grown. This organism grew readily on blood agar and was identified by cultural and morphologic characteristics.

Autopsy.—The body was that of a normally developed and well nourished infant. No lesions of the skin were seen. A small amount of dark frothy fluid oozed from the nostrils. There were no palpable superficial nodes.

The peritoneal cavity contained about 200 cc. of slightly turbid yellow fluid, and there was a fresh generalized fibrinous exudate over the peritoneal surfaces of the organs, with strands of fibrin adherent to the serosa of the intestines.

Each pleural cavity contained approximately 100 cc. of clear straw-colored fluid. There was no fibrinous exudate, and the pleural surfaces were smooth.

Neither the mediastinal lymph nodes nor the tracheobronchial nodes were enlarged.

The heart was grossly normal.

The pleura was smooth and free from exudate. The bronchi contained a small amount of frothy fluid. At the apex of the right lung a small area of tissue felt firmer than normal but differed little in color from the surrounding pulmonary tissue. The cut surface of the lung showed widely scattered, indefinitely outlined small reddish gray patches of bronchopneumonia. No large areas of consolidation other than that at the apex of the right lung were recognized.

The liver weighed 390 Gm. It extended about 3.5 cm. below the costal margin. It was pinkish yellow, and on section the lobules were distinctly marked out with yellow peripheral and red central zones.

The spleen was enlarged, weighing 70 Gm. Its capsule was covered with a fresh fibrinous exudate. On section, the malpighian bodies were distinct, and in addition there were many small yellow-gray areas of various sizes scattered throughout the spleen, which were soft and set off by a background of dark red pulp. These resembled disseminated tubercles except that they were a little softer than tubercles usually are.

The pancreas, adrenals and genitourinary organs were normal.

The stomach and duodenum contained a dark brownish fluid. The mucosa showed no injection, and there was no ulceration. The jejunum was normal. In the ileum, beginning in the proximal portion and becoming larger and more numerous as the cecum was approached, there were ragged ulcerations of the mucosa. These ulcers varied in diameter from a few millimeters to 1.5 cm., and had ragged outlines with raised borders. Many were definitely limited to swollen hyperplastic grayish red Peyer's patches. Others were smaller, isolated, surrounded by a raised border and not so obviously situated in lymphoid tissue.

In a few of the lesions the ulceration went through the submucosa and at least part of the muscularis so that only the serosa seemed to separate the lumen of the intestine from the peritoneal cavity—although none of the ulcers had perforated. At the ileocecal valve the lymphoid tissue was extremely hyperplastic and encircled the intestine. It contained many ragged ulcerations. On the serosal surface over many of the ulcers one could see dilated lymphatics marked out by gray interlacing lines such as occur on the serosa over tuberculous ulcers of the intestine.

The appendix was not inflamed. It contained no hyperplastic lymphoid tissue nor was its mucosa involved in the ulcerative process seen in the ileum.

All the mesenteric nodes in that part of the mesentery attached to the ileum were large and soft, varying in size up to 1.5 cm. in diameter. On section one

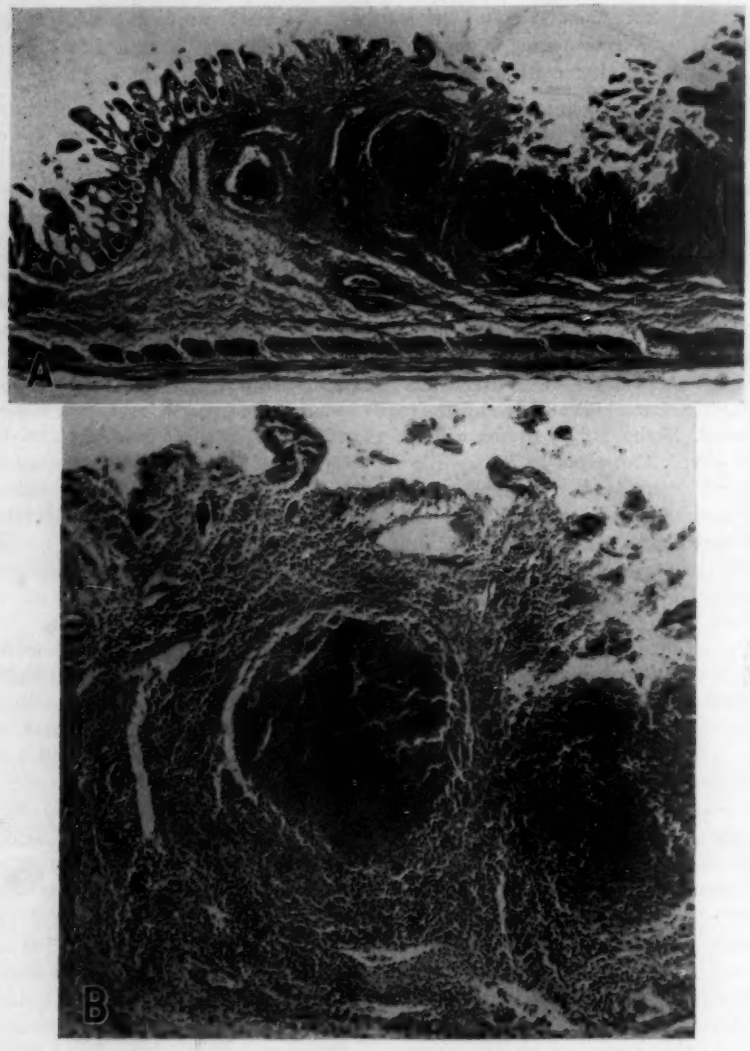

Fig. 1.—*A*, ulcer of the ileum, involving one of Peyer's patches. *B*, high power magnification of a necrotic lesion at the base of the ulcer seen in *A*.

found them replaced by areas of yellow-gray caseous material and a little softer than the usual caseous tuberculous lymph node. Where they were not caseous they were somewhat hyperemic.

Other lymph nodes were not grossly involved in the process and were not increased in size.

Bacteriologic Studies.—At the time of the autopsy the gross lesions suggested the possibility of primary intestinal tuberculosis of bovine origin. For

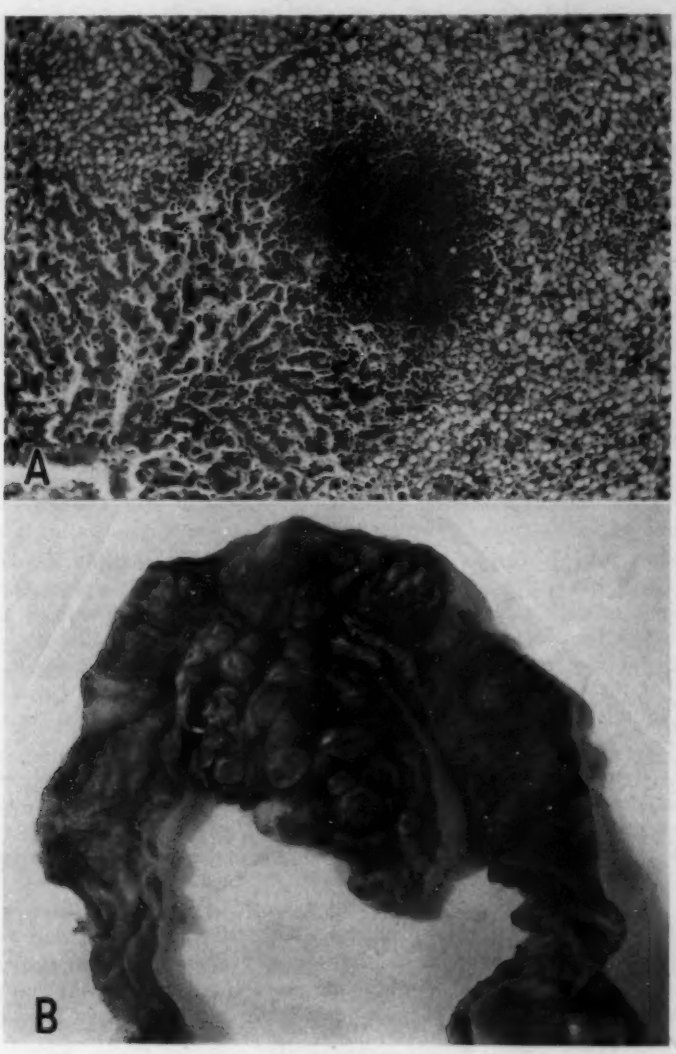

Fig. 2.—*A,* focal necrotic lesion in the liver. *B,* terminal part of the ileum, showing ulcerations and regional lymph nodes.

this reason material from a mesenteric lymph node ground up in saline solution was injected into 2 guinea pigs and two rabbits. Four days later the guinea pigs died, and on the fifth day the rabbits died. In the spleen, liver and lymph nodes of each of these animals there were foci of necrosis, more extensive in the rabbits

than in the guinea pigs. Tularemia was then suspected, although the history and age of the patient made this seem almost incredible. Ground-up splenic tissue from one of the rabbits was cultured and also rubbed on the shaved abdominal skin of two more guinea pigs. These also died in four days and showed similar lesions. Because the cultures were contaminated, Bacillus tularensis was not isolated on the first attempt, but on repeated attempts it was twice cultured on dextrose-cystine-blood agar from the splenic tissue of the guinea pigs and definitely identified by agglutination with specific antiserum. In the meantime the organism was passed in series through 5 guinea pigs by rubbing the shaved abdominal skin with splenic tissue.

A minute gram-negative organism could be demonstrated in sections of tissue from the guinea pigs, but none was demonstrable in sections of human tissue.

Serum was obtained from all the members of the infant's family, but none gave positive agglutination for B. tularensis.

Microscopic Examination.—The heart muscle, kidney, pancreas and adrenals were normal. The thymus was atrophic.

A section from one of the ulcers in the ileum showed a crater extending deep into the submucosa. It was lined by an eosin-stained necrotic layer of tissue, in which there were pyknotic and fragmented cell nuclei. A little farther from the necrotic edge of the ulcer, where the individual cells were recognizable, one found an infiltrate of wandering cells, which were predominantly mononuclear, with a smaller number of polymorphonuclear leukocytes and lymphocytes. Both layers of muscle were heavily infiltrated with large mononuclear cells, lymphocytes and an occasional polymorphonuclear leukocyte. A few cells extended into the edematous subserous tissue. There was no exudate on the serous surface.

Another, larger ulcer was definitely within one of Peyer's patches. The surface of the patch was partly ulcerated and covered with necrotic tissue, fibrin and polymorphonuclear leukocytes. In places the ulceration could be seen to have occurred in a focus of mononuclear cells of epithelioid character. In other places, where the surface was still intact, there were multiple large focal accumulations of cells of the same type, in the center of which extensive necrosis had taken place. In this section the muscle was not so extensively involved, but a few lymphocytes penetrated as far as the subserous tissue.

The spleen showed many focal necroses of various sizes. In most of these one saw only the débris of nuclei, remnants of red blood cells and the eosin-stained débris of cell cytoplasm. There was usually no margin of reaction around the necroses. A zone of hemorrhage sometimes occurred about them. Occasionally, especially about the larger necroses, there was an outer zone of intact large mononuclear cells with abundant cytoplasm. Clumps of similar cells occurred in sinusoids. The pulp spaces were engorged with red blood cells, some of which had been phagocytosed by scattered large mononuclear cells. On the capsular surface there was a thin layer of fibrin and necrotic cellular débris. The malpighian bodies were fairly large. The position of the necroses had no fixed relation to them.

The liver cells in zones about the portal spaces contained large fat droplets. There were scattered, indefinitely outlined small focal necroses. They had no relation to any part of the lobular structure. They resembled typhoid necroses or exudative miliary tubercles. In foci where the necrosis was not so advanced, one saw collections of polymorphonuclear leukocytes, large mononuclear cells and a few lymphocytes filling the sinusoids of the liver and compressing the surrounding hepatic cells. The necrosis involved both these infiltrating cells and the

surrounding liver cells. A few small branches of the hepatic and portal veins were filled with plugs of necrotic cells.

Two sections of the adrenals showed several small focal necroses similar to those seen in the liver.

In sections taken from one of the small peripancreatic lymph nodes and a large mesenteric node there was infiltration by large mononuclear cells. Many of these contained phagocytosed nuclear fragments and red blood cells. Indefinitely outlined foci of necrosis occurred in focal accumulations of these cells. In the larger node extensive areas of necrosis occurred without a limiting border of cellular reaction. In this node the necrosis extended through the capsule in several places.

Sections of the tracheobronchial nodes were slightly hyperemic. The peripheral sinus of one node contained red blood cells, polymorphonuclear leukocytes and a few large mononuclear cells. There was nothing suggestive of a reaction to B. tularensis.

There was patchy bronchopneumonia, which was most extensive in the upper lobe of the right lung. The walls of the larger bronchi were thicker than normal. They showed an increase of peribronchial connective tissue and of mononuclear wandering cells. There was no reaction about the smaller bronchioles or in the alveolar walls. Many of the capillaries were dilated. In groups of alveoli there was an exudate of red blood cells, polymorphonuclear leukocytes and large mononuclear cells. The polymorphonuclear leukocyte was the predominating cell. In places, the exudate was necrotic but this seemed to be associated with aspiration of material containing large numbers of gram-positive bacteria.

COMMENT

The microscopic lesions in the spleen, liver, abdominal lymph nodes and intestine were characteristic of tularemia although on the whole they showed more extensive necrosis and less proliferative reaction than has been usually described in cases in man.

The inflammatory process in the lungs appears to have been an ordinary pyogenic pneumonia. The influenza bacillus cultured from the blood shortly before death may have played a primary etiologic role in this process. There seems to be little doubt that the organism grown from the blood was the influenza bacillus. It grew readily on blood agar and was identified by its morphologic and cultural characteristics. The pneumonic exudate did not seem old enough to have been present since the onset of the illness two weeks before death, although there were said to be physical signs of a pneumonia at that time. The absence of involvement of the tracheobronchial nodes or of any of the thoracic nodes seems to be good evidence against the tularemic character of this infection of the lungs, aside from the nature of the inflammatory process itself.

The gross and microscopic observations, with the conspicuous intestinal ulcers and the lymphoid hyperplasia in the intestine and the correspondingly large caseous mesenteric nodes, without any superficial primary lesion and without involvement of lymph nodes outside the abdominal cavity, suggest strongly that the intestinal lesions were primary. One is then led to speculate on the possibility that the route of infection was through the intestinal tract following ingestion of infected material, although nothing in the history lends much help in establishing this fact. From the history obtained the only possibility seems to be that

the child may have picked up something carried into the house or yard by the dogs, which were allowed considerable freedom.

In an extensive study of the pathologic aspects of tularemia Lillie and his associates [1] reviewed the reported cases of tularemia in which autopsies were made—38 in all. In this group the gastrointestinal tract was not described or was reported as normal in all but 11 cases. In 5 of these congestion and petechial hemorrhages in the mucosa of the stomach and intestines were the only lesions reported in the gastrointestinal tract. In 5 others hyperplasia of the lymphoid tissue was described. In 4 of the latter group there were superficial ulcers in various parts of the gastrointestinal tract. In one, reported by Archer, Blackford and Wissler,[2] there were small superficial ulcers in the stomach only; in another, reported by Beck and Merkel,[3] there were shallow ulcers of the pylorus, duodenum, ileum and colon; in a third, reported by Fetterman and Lerner,[4] shallow ulcers of the ileum were described, and in the fourth case, reviewed and reported for the first time by Lillie and his associates, there were shallow ulcers in the cecum. In another case, reviewed by Lillie and his associates and previously unreported, ulcerations of an inflamed appendix were described, but no section of the appendix was available for their review of the case.

No other autopsy reports describing intestinal lesions in tularemia have appeared in the literature since the review mentioned, which was published in 1937.

In the present case the ulcerations were much more extensive and penetrated deeper into the intestinal wall than in any of the previously reported cases. Microscopically, they were quite consistent with the known cellular reaction to B. tularensis.

In 2 instances among the cases reviewed by Lillie and his associates, ingestion was considered as the possible mode of infection. One only of these, that reported by Beck and Merkel,[3] was characterized by the presence of intestinal lesions. Greatly involved abdominal lymph nodes were also noted in this case. In the other, that of Amoss and Sprunt,[5] intestinal ulcerative lesions were not present, and the most conspicuous lesions were those in the lungs and tracheobronchial lymph nodes.

In the literature there are frequent reports of tularemia in children, who are usually of an age when trapping of animals is popular. However, the only recorded fatalities in children under 6 years of age are those in 2 cases reported by Crawford [6] and another by Freese, Lake and Francis.[7] Crawford reported the infection of 7 members of a family from eating a rabbit which had been found dead. Three of the children, including a baby girl 14 months old, and two boys aged 2 and

1. Lillie, R. D., and others: The Pathology of Tularaemia, Bulletin 167, National Institute of Health, United States Treasury Department, Public Health Service, 1937.

2. Archer, V. W.; Blackford, S. D., and Wissler, J. E.: J. A. M. A. **104**: 895, 1935.

3. Beck, H. G., and Merkel, W. C.: South. M. J. **28**:422, 1935.

4. Fetterman, G. H., and Lerner, H.: J. Lab. & Clin. Med. **21**:1157, 1936.

5. Amoss, H. L., and Sprunt, D. H.: J. A. M. A. **106**:1078, 1936.

6. Crawford, M.: J. A. M. A. **99**:1497, 1932.

7. Freese, H. L.; Lake, G. C., and Francis, E.: Pub. Health Rep. **41**:326, 1926.

10 years, respectively, died. Freese, Lake and Francis reported the death of three members of a family, including a boy aged 2 years. In this instance the mode of infection was uncertain, but wild rabbit had been a frequent article of diet in the family. No autopsies were performed on these children. The youngest reported patient with this disease coming to autopsy, a 9 year old boy, was reported by Archer, Blackford and Wissler.[2]

SUMMARY

A case of tularemia in an infant 1 year of age is reported. The intestinal lesions, including hyperplasia of lymphoid tissue and deep ulcerations of the ileum, were more extensive than in any previously reported case. Although no definite information as to the mode of infection could be obtained, the extensive lesions in the intestinal tract and the regional nodes, without a primary superficial lesion, tularemic pneumonia or involvement of lymph nodes outside the abdominal cavity, have led us to suspect that the infective material was ingested.

CONGENITAL ABSENCE OF THE GALLBLADDER

G. KASTEN TALLMADGE, M.D., PH.D., MILWAUKEE

The cadaver of a white man 51 years of age showed congenital absence of the gallbladder, of the cystic artery, and of a part of the cystic duct, and a very rare anomaly of the hepatic artery. The man had died of chronic myocarditis and had shown no jaundice or other sign or symptom referable to the liver or to the biliary tract. The entire cadaver was scrutinized minutely, within as well as without, for traces of a surgical operation, but none could be found. And indeed the nature of the abnormalities observed was such as to make it certain that they were the result of embryonic maldevelopment and not of surgical procedures.

The liver showed no evidence of disease nor was it stained with bile. It did not deviate from the normal in shape, size or configuration except that the quadrate lobe was so poorly developed as to be concave and barely distinguishable, while the papillary process of the caudate lobe was somewhat larger than normal. The relative positions of the vessels of the porta were normal. The hepatic duct was 3.2 cm. long and greatly dilated, having an average diameter of about 1.8 cm. Its wall, on the other hand, averaged only 1.5 mm. in thickness. The cystic duct was 2 cm. in length and 3 mm. in diameter. Its lumen was patent where it entered the hepatic (or common) duct, of a size which would admit nothing thicker than an ordinary pin, and about 2 mm. in depth. The rest of the cystic duct was represented by a thin cord of fibrous tissue with a few poorly developed smooth muscle fibers. The lumen was blind, and, as was said, the gallbladder was absent. The common duct continued the width of 1.8 cm. of the hepatic duct until it reached the posterior surface of the duodenum, then it narrowed very rapidly to nearly normal size. The duodenal papilla was somewhat larger than normal.

The celiac artery had three branches. The left gastric and the lienal arteries were normal in size and course. The hepatic artery was not present; in its place was the gastroduodenal artery, which, descending toward the pylorus, gave off the right gastric artery. No artery from the celiac artery or from any of its branches went to the liver. The hepatic artery (in this case, the arteria hepatica propria) arose as the first branch of the superior mesenteric artery, ran upward in front of the portal vein and, near the porta of the liver, divided into a right and a left branch. The cystic artery, normally arising from the right branch, was entirely absent.

Adachi [1] showed that an observation of this type is one of true origin of the hepatic artery (as distinguished from an anomalous right accessory hepatic artery) from the superior mesenteric artery, since the artery lies ventral, not dorsal, to the portal vein. This is, therefore, the third case which has been reported (Lyssenkow's [2] was the second), and the first to be reported in the western hemisphere.

From the Department of Anatomy, Marquette University School of Medicine.

1. Adachi, B.: Das Arteriensystem der Japaner, Kyoto, University of Kyoto, 1928.

2. Lyssenkow, N. K.: Anat. Anz. **75**:184, 1932.

The interest in the arterial anomaly, however, is more or less academic. Of more practical interest is the congenital absence of the gallbladder. Stone [3] operated on a woman 54 years of age for gallstones. The stones were found, but they were in the hepatic and common ducts. No gallbladder was present, and the cystic duct was represented by "a papule very near the [usual] site of the cystic duct." Stone remarked that the most rigid search should be made for a gallbladder that is apparently absent, since in cases in which inflammation has occurred the organ may be lost from view, retracted, shriveled and shrunken in a mass of fibrous tissue and easily mistaken for one of the ducts. He himself more than once opened the common and hepatic ducts before he could find the gallbladder via the cystic duct. Kehr [4] reported a case occurring in an actor in whom several physicians had palpated gallstones, but at operation neither stones nor gallbladder could be found. He also reported a case in which the gallbladder was situated within the substance of the liver; this was supposed to have been proved by puncture. However, it is possible that the trocar entered a dilated hepatic duct and bile appeared, although intrahepatic gallbladder occurs.[5]

Kirshbaum [6] stated that congenital absence of the gallbladder and of the extrahepatic bile ducts is probably the second in order of frequency of rare anomalies of these organs. The presence of two livers is given as the rarest. The former he found in 1 of 3,669 autopsies at the Cook County Hospital, Chicago, from 1928 to 1934. Another group, cited by Danzis,[7] showed 6 cases of congenital absence of the gallbladder in 7,919 autopsies. The present case was the only one found in a series of 162 cadavers. Scammon [8] made a study of animals which normally have no gallbladder in postnatal life, but found absence of the gallbladder rare in man. He stated that in man the gallbladder alone may be lacking or more rarely both the gallbladder and the larger biliary ducts. This statement, however, seems to be open to debate. One must remember that a relatively large number of cases occur in infants and that the mortality in infants from congenital defects of the biliary system has never been determined. Danzis, in his excellent survey, collected 96 cases, 31 of which were observed in infants less than a year old; in most of these the diagnosis was made at operation; in a few, by necropsy. The number of cases actually occurring but remaining without diagnosis may be much larger among infants who die after a few days or weeks of life with melena, hemorrhage from the navel or other symptoms which Danzis found to be associated with this congenital defect.

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3. Stone, I. S.: Am. J. M. Sc. **135**:889, 1908.
4. Kehr, H.: Berl. klin. Wchnschr. **50**:511, 1913.
5. Walter, O. M., and Neiman, A.: Illinois M. J. **60**:478, 1931.
6. Kirshbaum, J. D.: Am. J. Dis. Child. **47**:1080, 1934.
7. Danzis, M.: Am. J. Surg. **29**:202, 1935.
8. Scammon, R. E.: Anat. Rec. **10**:543, 1916.

SUMMARY

On collecting all the available series (adding Mackmull's [9] series to those already mentioned), agenesis of the gallbladder was found in 12 of 18,350 cases, an incidence of 0.065 per cent.

One third of the reported cases have been found, at operation or at autopsy, to occur in children less than a year old. The number of additional cases which may occur in infants and especially in infants who die within the first five days of postnatal life, in which no diagnosis is made, can only be surmised but must be considerable.

A thorough search must be made for a gallbladder that is apparently absent, because the gallbladder may be shrunken, embedded in scar tissue, abnormally located or enclosed within the liver. It should be remembered that agenesis of the gallbladder is an anomaly of the first order of rarity.

9. Mackmull, G.: Ann. Surg. **91**:789, 1930.

Laboratory Methods and Technical Notes

THE SPALTEHOLZ CLEARING METHOD APPLIED TO THE DETERMINATION OF CARCINOMATOUS METASTASES IN REGIONAL LYMPH NODES

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It is of importance to determine with accuracy the presence or absence of metastatic involvement of regional lymph nodes in surgically resected specimens of carcinoma. Routine examination usually includes selection by gross dissection of the suspicious nodes and microscopic examination of these nodes for malignant cells. We feel, however, that this method has been inadequate and incomplete. Therefore, it is our custom to employ the following procedure in cases in which the regional lymph nodes have been reported without evidence of metastasis after the usual routine examination. These cases include those of carcinoma of any portion of the gastrointestinal tract and also those of cancer of the breast. The method is based essentially on the process of clearing tissues originally described by Spalteholz and applied by Westhues [1] in his studies of carcinoma of the rectum.

PROCEDURE

The specimen, which has already been fixed in Kaiserling solution 1, is washed in running water and then dehydrated in several changes of alcohol, working from 85 per cent up to absolute alcohol. The duration of each change is about two to three days. The specimen is then transferred to a solution of methyl salicylate U. S. P. and allowed to clear. The time required for complete clearing varies from a few hours to several days, depending on the thickness of the tissues and the degree of dehydration accomplished by the alcohol. When clearing is complete, the specimen is carefully searched for lymph nodes. These are seen as more or less opaque nodules, which stand out in contrast with the almost transparent fat tissue. Occasionally, some of the nodes become fairly clear, but with careful examination in a good light, accurate palpation and dissection, it is fairly simple to identify them. After the nodes have been dissected out, they are placed in absolute alcohol and then prepared in the usual manner for paraffin sections and staining with hematoxylin and eosin.

The results of our studies will be reported in detail in a future communication. It may be stated at the present time, however, that with the aid of this method we have been able to find more lymph

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From the laboratories of The Mount Sinai Hospital.

1. Westhues, H.: Die pathologisch-anatomischen Grundlagen der Chirurgie des Rektum Karzinoms, Leipzig, Georg Thieme, 1934.

nodes in our specimens than could be discovered even with meticulous dissection. Even nodes as small as the head of a pin can be easily identified and picked out for study. In a number of cases we have found metastatic foci in some of the very small nodes which were missed in the routine examination.

Thus more accurate data have been obtained on which to base studies of prognosis and mortality in relation to the involvement of regional lymph nodes. We recommend the routine use of this method in every case in which the original search has failed to reveal metastases in lymph nodes.

Notes and News

University News, Promotions, Resignations, Appointments, Deaths, Etc.—George W. McCoy, medical director of the United States Public Health Service, has been appointed professor of preventive medicine and public health in the School of Medicine of Louisiana State University.

Alexander Fraser, professor of pathologic histology in New York University from 1920 until his retirement in 1934, died Sept. 19, 1938, at the age of 69 years.

In the University of Iowa, Emory D. Warner has been promoted to the post of associate professor of pathology.

Sir Andrew Macphail, professor of the history of medicine at McGill University, Montreal, from 1907 until he retired in 1937, has died at the age of 74 years. He wrote the official history of Canada's medical services in the world war and a biography of John McCrae, physician, who wrote the war poem "In Flanders Fields."

Horst Oertel, Strathcona professor of pathology in McGill University and pathologist to the Royal Victoria Hospital, Montreal, having reached the age limit, was retired at the end of the last academic session.

Society News.—Charles F. Branch has been elected president (for one year) and Granville Bennet secretary (for three years) of the New England Pathological Society.

Wright Clarkson has been elected president and Eugene R. Whitmore, Washington, D. C., secretary of the American Society for the Study of Neoplastic Diseases.

The New York State Society of Pathologists has adopted a constitution and elected officers as follows: W. S. Thomas, president; A. V. St. George, vice president; M. J. Fein, secretary-treasurer. Committees have been appointed to work toward the improvement of the economic status of pathologists in the state of New York.

At the annual meeting in San Francisco last June, the American Society of Clinical Pathologists elected the following officers: president-elect, L. W. Larson; vice president, W. Cummins; secretary-treasurer, A. S. Giordano. The president for the coming year is T. B. Magath.

The Fourth International Congress of Comparative Pathology will be held in Rome, May 15-20, 1939. There will be sections on human medicine, veterinary medicine and phytopathology.

The twenty-third annual meeting of the American College of Physicians will be held in New Orleans, March 27-31, 1939.

A New Medical Journal.—The first number of the *American Journal of Medical Jurisprudence* has appeared. The new journal, which is edited by Frederick C. Warnshuis, is published monthly by the American Medico-Legal Association, 137 Newberry Street, Boston, Mass.

Abstracts from Current Literature

To Save Space the Original Titles of Abstracted Articles Sometimes Are Shortened

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Experimental Pathology and Pathologic Physiology

Hypertension Produced by Constriction of the Renal Artery in Sympathectomized Dogs. N. E. Freeman and I. H. Page, Am. Heart J. **14**:405, 1937.

Complete sympathectomy, in 7 dogs, did not prevent the development of hypertension from compression of the renal arteries. The level of the hypertension was not materially affected during the course of total sympathectomy. There was no evidence of an increase in medulliadrenal secretion. An injection of ergotamine tartrate (3 mg.) produced a slight further increase in the hypertension, but this dose was insufficient to reverse the pressor effect of epinephrine (0.3 cc. of a 1:1,000 solution intravenously). Denervation of the heart combined with total sympathectomy did not influence the course of the hypertension. The plasma volume may not be increased as the hypertension develops. These observations suggest that the known physiologic factors which normally control the level of arterial pressure are not etiologically significant in the genesis of experimental hypertension produced by compression of the renal arteries.

From Authors' Summary.

Effects of Coal Smoke of Known Composition on the Lungs of Animals. L. Schnurer and S. R. Haythorn, Am. J. Path. **13**:799, 1937.

An experimental method is described by which finely divided carbon particles in an almost pure state may be introduced into the alveoli of the lungs. Immediately after inhalations of the smoke the lesions of the lungs were those of nonoccupational anthracosis. The animals kept under the conditions of the animal house for from several months to a year after exposure to the smoke showed fibrous reactions about the carbon deposits, with the formation of collagen strands. The resulting pulmonary changes were analogous to those of a milder grade of bituminosis as seen in soft coal miners. The method provides a way of preparing lungs for experimental study of the actual relation of carbon deposits to the common respiratory infections.

From Authors' Summary.

Fatty Infiltration and Cirrhosis of the Liver in Depancreatized Dogs Maintained with Insulin. I. L. Chaikoff, C. L. Connor and G. R. Biskind, Am. J. Path. **14**:101, 1938.

Depancreatized dogs constantly present fatty livers at variable periods after the operation has been performed. Of 16 kept for from two and a half to five and a half years on an adequate diet and insulin, 8 showed more or less interlobular fibrosis of the liver associated with a hyaline or colloid degeneration of many cells and hyaline atrophy of peripheral cells. In 4 animals this was so pronounced, both grossly and microscopically, that the picture of a well advanced portal cirrhosis of the liver was present. By the time this severe cirrhosis had occurred, the fat content of the liver had returned to normal, and there was little histologic evidence that a markedly fatty liver had preceded the fibrosis. The sequence of events appears to be fatty infiltration, hyaline degeneration and atrophy of cells at the periphery of lobules and fibroblastic proliferation in orderly fashion, ending

with the typical hobnail appearance and fibrotic structure of cirrhosis. Necrotizing agents introduced from the outside, infection and extrahepatic biliary obstruction were excluded as causative factors.

FROM AUTHORS' SUMMARY.

COMPARATIVE EFFECTS OF NEUTRONS AND ROENTGEN RAYS ON THE WHOLE BODY. J. H. LAWRENCE and R. TENNANT, J. Exper. Med. **66**:667, 1937.

Irradiation of the whole bodies of mice with neutron rays in sufficient quantities leads to a clinical, bacteriologic and anatomic picture similar to that following roentgen irradiation. The mucosa of the small intestine and the lymphoid and hemopoietic tissues are the most radiosensitive. The mechanism of death after both forms of irradiation seems to be a combination of tissue destruction and enterogenous infection, the former predominating in the acute deaths after large doses. Aside from any possible delayed effects from exposure to small doses over a long period of time, concerning which there is no information, these changes after relatively large doses make it imperative that workers in laboratories generating neutrons protect themselves from exposure by screening. For the same amount of ionization measured by a small bakelite-walled thimble chamber, neutrons are more biologically destructive than roentgen rays. The average daily dose to those working with neutrons should not exceed one fourth of the tolerance dose accepted for roentgen rays. Whether daily doses of this magnitude over a long period of time will result in damage is not known. Also, if neutrons are tried therapeutically, normal tissue must be protected from undue irradiation.

FROM AUTHORS' SUMMARY.

EFFECT OF HYPERTROPHIC CARTILAGE ON BONE MARROW GROWTH. C. HUGGINS and K. M. SMITH, J. Exper. Med. **67**:41, 1938.

Intravenous injections of colloidal thorium dioxide were made in rabbits, and moderate amounts caused no significant changes in the cellular elements of venous blood. Thorium dioxide was held locally with great tenacity in the cells of the reticuloendothelial system in the bone marrow and showed little tendency to migrate despite drastic stimuli to the marrow in the form of anemia and plethora. In rabbits recently given injections thorium-laden macrophages abutted each cartilage column in the direction of growth where the cartilage was being removed. In marrow which had formed subsequent to the injections the macrophages were free from thorium, allowing recognition of the new tissue by roentgenologic and histologic technic as clear zones. In this way the growth pattern of marrow could be detected. The bone marrow increases in length principally in the region of hypertrophic cartilage at the metaphyses, and it is evident that the increase is facilitated by the macrophages, the primary function of which is the resolution of the wasted hypertrophic cartilage cells. Growth in thickness of the marrow occurs at the circumference of the marrow. In contrast to the zonal growth of marrow, growth of the reticuloendothelial tissues in the liver and spleen is chiefly interstitial.

FROM AUTHORS' SUMMARY.

STUDIES ON INFLAMMATION. V. MENKIN, J. Exper. Med. **67**:129, 145 and 153, 1938.

There is present in inflammatory exudates a factor which induces a prompt increase in the permeability of normal capillaries. Its liberation and presence in exudates offer a reasonable explanation for the mechanism of increased permeability of small vessels in injured tissue. A method for the isolation and purification of this permeability factor has been described. In its essential features, it consists of treating the exudate with pyridine and then with acetone. After separation of the protein fractions further purification can be obtained by prolonged extraction with butyl alcohol or by subjecting the acetone supernatant fraction to low temperature (—20 C.). The latter favors spontaneous separation of the active principle. The purified material is a crystalline, doubly refractive nitrogenous substance. The factor is evidently not a protein, yet it contains amino and carboxyl groups. It

gives a positive test for the presence of an indole nucleus in its structure (Adamkiewicz test). The active material is dialyzable; it is precipitated by concentrated ammonium sulfate. The present evidence indicates that it is an intermediary breakdown product of protein metabolism, probably belonging to the group of relatively simple polypeptides. Further studies are being conducted in an endeavor to determine its precise chemical structure.

A crystalline nitrogenous substance recovered from inflammatory exudates induces in cutaneous tissue a rapid migration of polymorphonuclear cells through the endothelial walls of small vessels. For the sake of convenience the tentative name "leukotaxine" has been proposed for this active substance which per se is capable of rapidly reproducing the basic sequences of the inflammatory reaction, namely, the increase in capillary permeability and the outward migration of polymorphonuclear leukocytes. Comparison of the effect of various inflammatory irritants (e. g., turpentine or aleuronat [an albuminoid substance and lecithin]) with that of leukotaxine indicates that the migration of cells does not seem to be primarily referable to the initial increase in vascular permeation. Furthermore, leukotaxine is definitely chemotactic. It induces an active migration of leukocytes into capillary glass tubes containing it. The relation of leukotaxine to the mechanism of cellular migration at the site of inflammation is pointed out.

Blood serum digested by trypsin yields split products which when injected into the skin of normal rabbits rapidly increase the permeability of small cutaneous vessels. This is likewise followed in the affected tissue by prompt local migration of polymorphonuclear leukocytes. The tryptic digest of blood serum can be purified by methods previously described. The resulting crystalline substance alters the permeability of the capillary wall and favors local migration of leukocytes in a manner similar to leukotaxine proper. The present observations indicate that leukotaxine or a substance having at least identical biologic properties appears to have been formed by proteolytic enzymatic action on an otherwise relatively inactive blood serum. This finding serves as additional evidence in support of the view that the leukotaxine recovered from inflammatory exudates is probably an intermediary product of protein catabolism. FROM AUTHOR'S SUMMARIES.

IRON METABOLISM IN EXPERIMENTAL ANEMIA. P. F. HAHN and G. H. WHIPPLE, J. Exper. Med. **67**:259, 1938.

In anemia in dogs due to loss of blood the term "available iron as determined by the dipyridyl test" has no physiologic significance. Iron salts (100 per cent available by the dipyridyl test) given in optimal dosage (560 mg. every two weeks) will cause a net production of from 50 to 55 Gm. of hemoglobin above the control base line in anemic dogs. This means that an iron salt which is rated as 100 per cent available by the dipyridyl test is only 35 per cent available physiologically. Available iron (dipyridyl) simmers down to iron not in the form of hematin compounds. The absorption of this "available iron" is conditioned by a great variety of factors, many unknown at this time. Iron is indeed an elusive sprite whose availability or comings and goings cannot be determined in dogs by dipyridyl—perhaps only in part by studies of absorption and excretion. Liver contains available iron (dipyridyl) but also organic factors influencing the regeneration of hemoglobin in anemia, as liver ash has only about 50 per cent of the potency of whole liver. One can readily dissociate iron from other potent factors in various tissues. Fractions of heart, liver, spleen and kidney may contain very little iron yet cause much regeneration of hemoglobin in anemic dogs. No investigator has reported any condition of copper deficiency in man or dog. In fact, in anemia copper is usually above the normal concentration in the liver. It is unlikely, therefore, that in experimental anemia in dogs and in the various anemias of man, any significance attaches to the intake of copper. FROM AUTHORS' SUMMARY.

INCREASED SUSCEPTIBILITY TO HEMOLYSIS BY INDOL IN DOGS FED DEFICIENT DIETS. C. P. RHOADS, W. H. BARKER and D. K. MILLER, J. Exper. Med. **67**:299, 1938.

Indole is more hemolytic when there is a dietary deficiency than when the diet is normal. The hemolytic effect can be abolished by supplementing the deficient diet with liver extract, curative of pernicious anemia in man. The hemolysis affects all hemoglobin-containing cells, including reticulocytes. Repair of the anemia resulting from the administration of indole in the presence of a dietary deficiency represents cessation of the hemolytic process. An abnormally low rate of production of erythrocytes may well be a factor in the production of the anemia.

FROM AUTHORS' CONCLUSIONS.

Pathologic Anatomy

PATHOLOGY OF TULAREMIA IN MAN. R. D. LILLIE and E. FRANCIS, Bulletin 167, National Institute of Health, 1937.

An account of the pathologic anatomic and histologic changes in tularemia in man is presented, based on published and unpublished observations in 32 autopsies and on 52 surgical specimens, and more particularly on histologic material in 17 published and 9 unpublished reports of autopsies and 13 published and 7 unpublished records of surgical cases.

In man, as in animals, two concurrent actions may be discerned: (1) the killing or necrotizing effect of the causative agent, and (2) a reaction of reticulo-endothelial-monocyte-epithelioid cell type. The polymorphonuclear leukocyte plays little part in the reactive phenomena except in the presence of secondary infections with pyogenic micro-organisms. In early lesions and in those proximal to the primary lesions necrosis tends to dominate and involves preexisting fixed tissues as well as cellular exudates. In later or more distant lesions the reactive phenomena dominate, and necrosis may be of slight extent or absent. Finally, old necrotic lesions become encapsulated, first by monocytes and epithelioid cells and later by fibrous tissue. Giant cells occur with appreciable frequency only in late lesions.

No such accumulation of Pasteurella tularensis has been observed in tissue cells of man as occurs in mice, cottontail rabbits, jack rabbits, cotton rats and some opossums and guinea pigs, and the few reports of the demonstration of P. tularensis or organisms resembling it in lungs and lymph nodes are not convincing.

A nodular, confluent nodular or pseudolobar, usually monocytic pneumonia with considerable tendency toward caseous necrosis of exudate and septums has been present in about 70 per cent of the cases, and when the total of pleural, focal and pneumonic involvement is taken the percentage of pulmonary involvement is about 85 per cent.

The spleen is often enlarged (average, 340 Gm.; extremes, 110 and 950 Gm.). In about 70 per cent of the cases it contains focal lesions—focal necroses in the earlier stages, granulomas in the late ones.

The liver has shown focal lesions in about 55 per cent of the cases—necrosing monocytic nodules in most cases and definitely granulomatous lesions in the cases in which the disease is in a late stage.

Focal lesions have not thus far been found in the bone marrow in man.

A peritoneal involvement, either localized to the splenic area or more or less generalized, occurs in about 40 per cent of the cases and is characterized by fibrinous or fibrinocaseous exudation and infiltration by lymphoid cells and monocytes, with or without foci of necrosis.

The regional lymph nodes are regularly enlarged in ulceroglandular, glandular and oculoglandular cases. One case in which there was an ulcer of a finger is recorded in which regional nodes were specifically noted as not enlarged. Usually they present focal or diffuse caseous necrosis, sometimes suppuration. In all types of cases the mediastinal nodes are usually enlarged and focally caseous or some-

times suppurating. In regional nodes in recent cases, coagulative to caseous necrosis dominated; distant nodes often showed sinus reticuloendotheliosis and exudation of macrophages, with or without necrosis. Late lesions presented epithelioid cell margination, Langhans' giant cells and, later, fibrosis. Giant cells are rarely seen in fatal cases.

Focal lesions probably assignable to tularemia have occasionally been recorded as observed in the kidneys, intestines, brain and meninges. The renal lesions were focal necroses with mononuclear and plasma cell margination; the intestinal lesions, necrosis and ulceration in lymphoid follicles, comparable to the lesions in the lymph nodes. The lesions in the brain were hemorrhagic or caseous focal necroses. The meningeal reaction was a necrosing nodular and diffuse monocytic meningitis.

[The same bulletin contains articles by the same authors, associated in two instances with R. R. Parker, on tularemia in various mammals and birds.—Abstracter.]

FROM AUTHORS' SUMMARY.

CYSTIC CHANGES IN THE ENDOMETRIUM. L. M. RANDALL and W. E. HERRELL, Surg., Gynec. & Obst. **65**:666, 1937.

Microscopic examination of 28 specimens of endometrium revealed that cystic changes occurred in all phases of the menstrual cycle. When cysts occur in the proliferated phase there is often accompanying proliferation or so-called hyperplasia of a greater degree than occurs normally. A polypoid endometrium is usually increased in thickness, although the microscopic picture of proliferation remains the same. On the appearance of the differentiative phase, this proliferation is less noticeable, but the cystic changes persist. The microscopic observations appear to correlate with the clinical history. Atypical uterine bleeding was not present in any case in which a well differentiated endometrium was associated with cystic dilatation, but such bleeding was not infrequent when cysts were found in the early differentiative phase. It was more frequent still in cases in which a cystic endometrium occurred in the late proliferative phase of the menstrual cycle. The essential difference in these specimens of endometrium is the degree of differentiation which must exist because of a difference in the activity of the hormone of the corpus luteum. The authors feel that microscopic evidence does not favor the concept that the function of the corpus luteum is on an all or none basis. Endometrial cysts are very common in women at the beginning of the menopause, when the first phase of ovarian failure is commencing, and the condition is essentially the same as that among younger women with hypofunction of the corpus luteum.

WARREN C. HUNTER.

MEGAKARYOCYTIC MYELOSIS WITH OSTEOSCLEROSIS. T. F. HEWER, J. Path. & Bact. **45**:383, 1937.

The case is described of a man of 59 years whose condition was diagnosed clinically as splenic anemia and who had tumor-like foci of myeloid tissue with many megakaryocytes and a large proportion of hemocytoblasts and primitive erythroblasts in the spleen, simple hyperplasia of a more mature type in the vertebral marrow, osteosclerosis of the upper third of the femur and anemia of a secondary type. Similar cases have been described by French authors under the name of *splénomégalie myéloide mégacaryocytaire amyélocythémique*. The osteosclerosis observed in the present case and in some of those recorded in the literature appears to be of secondary importance and may be regarded as a local connective tissue response to the hyperplastic marrow. It is concluded that the condition is myelosis affecting the spleen and to a less extent the bone marrow, which may be due to lack of some factors necessary for further development. A plea is made for repeated examinations of the blood, splenic puncture, the use of epinephrine to force myeloid cells from the splenic sinusoids into the general

circulation, where they may be discovered, and careful roentgenologic examination of the bones in all conditions diagnosed clinically as splenic anemia and a more complete examination of the bone marrow at autopsy in such cases.

FROM AUTHOR'S SUMMARY.

THE BLOOD SUPPLY OF NEWLY DEVELOPED EPITHELIAL TISSUE IN THE LIVER. R. D. WRIGHT, J. Path. & Bact. **45**:405, 1937.

The blood vessels of 5 livers in which regeneration of liver tissue was apparent and of 15 livers in which secondary carcinoma was present were given an injection of an opaque substance, with the following results: 1. All regenerated hepatic parenchyma showed vessels of the sinusoidal type, with an efferent supply from the portal vein. Those areas which showed hyperplastic bile ducts showed also the development of a rich supply of vessels to these structures from the hepatic artery. It appears from this that the type of vessel normally associated with each of these epithelial tissues is activated to supply the corresponding type of newly developed tissue and that, associated with this vascular growth, there is the appropriate relation between the vasculature and the fibrous stroma. When a fibrovascular stroma develops in secondary carcinoma in the liver, the afferent blood supply to these vessels is from the hepatic artery. The main venous drainage from secondary carcinoma is into the portal veins.

FROM AUTHOR'S SUMMARY.

THE GROSS ANATOMY OF THE PARATHYROID GLANDS. J. R. GILMOUR, J. Path. & Bact. **46**:133, 1938.

The observations in a consecutive series of 428 dissections giving 1,713 parathyroid glands, established to be such by microscopic examination, are analyzed, and references to rare conditions found in subsequent dissections are added. The features noted were the number of glands in different subjects, their position and relation to other tissues, the size in age groups of those within the limits of normal weight and the variations in morphologic character and in color. A short description of the technic for their discovery in the cadaver is given as an appendix. Stress is laid on the embryonic history of the glands, which consequently are divided into those derived from the third pharyngeal pouch (parathyroids III) and those from the fourth (parathyroids IV), and it is shown that the course taken by the glands during the development of the embryo and their close association with the thymuses derived from the same pouches (thymus III, thymus lobules III and thymus IV) explain their permanent positions and relations and are therefore of the greatest practical importance in discovering them. The method of determining the pouch of origin is given. It is shown that parathyroids III are usually but not invariably the lower parathyroids.

FROM AUTHOR'S SUMMARY.

DEPOSITION OF AMYLOID IN THE WALL OF THE URETER. G. LEHMANN, Zentralbl. f. allg. Path. u. path. Anat. **68**:209, 1937.

Amyloid infiltration of the ureter was encountered in the body of a woman who died of hepatic coma accompanying cirrhosis of the liver. The amyloid was found in the lower two thirds of the left ureter and distended the wall to three times its usual thickness. It occurred in the inner half of the muscle layer, membrana propria and precapillary vessels. In addition to a rather homogeneous involvement, nodules occurred at irregular intervals. The overlying mucosa was flat and heavily invaded by lymphocytes. Foreign body giant cells were numerous at the borders of the amyloid accumulations, whereas calcification and bone formation were more deeply situated.

GEORGE J. RUKSTINAT.

TWO FURTHER CASES OF ACTIVE SYPHILITIC VALVULITIS WITH PRESENCE OF SPIROCHETES. H. ŠIKL and K. RASKA, Časop. lék. česk. **76**:793, 1937.

Two cases were described by the authors in a previous paper; in the present paper two new instances of gummatous involvement of the aortic semilunar valves have been added. In both, spirochetes were demonstrated in the diseased cusps. In the first case, concerning a 40 year old man, the dorsal aortic valve showed a rather extensive defect, due solely to gummatous necrosis; there were no signs of complicating bacterial endocarditis. The second case was in a man aged 60. Here the left aortic cusp was affected severely with gummatous lesions, whereas the remaining two cusps showed only fibroelastic thickening of the free margin without signs of necrosis or of recent inflammation. A rheumatic origin of the last-mentioned changes could not be excluded with certainty.

FROM AUTHORS' SUMMARY.

HISTOPHYSIOLOGIC AND ENDOCRINE INTERRELATIONSHIP OF THE ANTERIOR LOBE OF THE PITUITARY. S. FRANK, Acta path. et microbiol. Scandinav. **14**:339, 1937.

The results of the experiments reported here support the view that acidophilic and basophilic cells represent different secretory or functional stages of the cells of the anterior lobe of the pituitary.

Microbiology and Parasitology

PRESERVATION OF SPIROCHAETA PALLIDA AND TREPONEMA PERTENUE IN THE FROZEN STATE. T. B. TURNER, J. Exper. Med. **67**:61, 1938.

A simple method for freezing and maintaining tissue specimens in a mixture of solid carbon dioxide and 95 per cent ethyl alcohol at a temperature approximating —78 C. is described. When frozen and maintained at this temperature, Spirochaeta pallida and Treponema pertenue exhibited on thawing normal structure and motility, and their virulence for rabbits was not appreciably altered after periods of at least one year. This observation was made with a number of different strains of each organism. The infectivity of material in which treponemes or spirochetes were scant was maintained as well as that of material in which they were abundant. At temperatures of —10 and —20 C. the spirochetes did not survive as long as two months. Death of these organisms occurred not at the time of freezing but during the period of maintenance. Treponemes and spirochetes did not commonly survive freezing and desiccation; although one lot of dried material which contained S. pallida was infective for rabbits one day after desiccation. The viruses of human influenza, yellow fever and spontaneous encephalomyelitis of mice when frozen and maintained at —78 C. showed substantially the same titer after six months as before freezing. Certain practical applications of the method are suggested.

FROM AUTHOR'S SUMMARY.

INFLUENCE OF HOST FACTORS ON THE NEURONAL INVASIVENESS OF THE VIRUS OF VESICULAR STOMATITIS. A. B. SABIN and P. K. OLITSKY, J. Exper. Med. **67**:201 and 229, 1938.

The resistance of old mice to the virus of vesicular stomatitis peripherally inoculated appears to be the result of (*a*) changes produced by age, not in the whole animal, but in certain specific isolated structures, and (*b*) the special mode of dissemination of peripherally injected virus. The equal susceptibility of young and old mice to intracerebrally inoculated virus can be explained by the observation (*a*) that virus injected in this way spreads primarily in an open system (the lesions being distributed almost entirely in contiguity with the ventricles and their extensions) and (*b*) that in the old animal the central nervous system as a whole, although more resistant to neuronal necrosis, is still lethally affected

by the multiplication of the virus. After peripheral inoculation the evidence points to progression of the virus in a closed system of neurons and their processes, at least in the stage preceding neuronal necrosis, the distribution of lesions in the central nervous system depending on the central connections of the primary neurons connected with the inoculated site. In old mice the inability of the virus to involve the terminal processes or cell bodies of the neurons at the site of inoculation (as after intramuscular or intraocular injections) or to spread to other neurons along the chain in the central nervous system (as after nasal instillation) prevents the development of the clinically apparent and fatal disease.

Peripheral inoculation of the virus of vesicular stomatitis is constantly followed by myelitis or encephalitis in young mice but not in young (or old) guinea pigs. The cause of this variation was elucidated by investigating the fate of virus inoculated by a number of different routes. Direct intracerebral injection of minimally infective amounts of virus was found to be equally fatal for young mice and young guinea pigs, indicating that the central nervous system as a whole is as easily injured by the virus in one species as in the other. The events following nasal instillation of the virus varied in young and old guinea pigs. While there appeared to be a transitory multiplication of the virus in the nasal mucosa of both young and old animals, the central nervous system was regularly invaded only in the young.

FROM AUTHORS' SUMMARIES.

CULTIVATION OF THE RICKETTSIAS OF ROCKY MOUNTAIN SPOTTED FEVER IN VITRO. I. A. BENGTSON, Pub. Health Rep. **52**:1329, 1937.

The rickettsias of Rocky Mountain spotted fever have been cultivated through 15 passages in modified Maitland mediums in which as tissue the chorioallantoic membrane of chick embryos and guinea pig tunica were used. Substitution of Baker's for Tyrode's solution brought about an increase in the number of rickettsias in mediums containing chick embryo tissue and also gave good results in mediums with guinea pig tunica. There was no loss of virulence in the rickettsias thus cultivated, both tissue and supernatant fluid causing typical symptoms in guinea pigs, followed by death.

FROM AUTHOR'S SUMMARY.

THE NUTRITION OF STAPHYLOCOCCUS AUREUS. P. FILDES and G. M. RICHARDSON, Brit. J. Exper. Path. **18**:292, 1937.

For growth of Staphylococcus aureus there must be included in the nutrients an organic sulfur compound containing actually or potentially a mercaptogroup (—SH). All mercapto or dithio compounds tested were active to a greater or less extent. Cystine was outstandingly active, giving visible growth at concentration below 10^{-6} molar. Methionine as a potential source of —SH acts only poorly on growth but has an accelerating influence on the growth produced by cystine. It is argued that the effect on growth of a mercaptocompound is not exerted directly but by virtue of its accessibility as a component in the synthesis of cystine. Differences are noted in the utilization of methionine and of homocysteine by staphylococci and by animals as reported in the literature.

FROM AUTHORS' SUMMARY.

PROPERTIES OF DIFFERENT FRACTIONS OF DYSENTERY BACTERIA. L. OLITZKI, J. LEIBOWITZ and M. BERMAN, Brit. J. Exper. Path. **18**:305, 1937.

In three different strains of dysentery bacteria (Shiga, Flexner and Y) the following factors were found: (*a*) A glycemic factor, present in the fractions P 1, C and Ha. It is also present in unpurified P 2, but after extraction of this fraction with 70 per cent alcohol it is no longer demonstrable. (*b*) A leukopenic factor, present in the fractions P 1 and P 2, to a lesser extent in C and not at all in Ha. (*c*) An endotoxic factor, present mainly in P 2 and to a small extent

in P 1 but absent from C and Ha. The Ha substances of all three strains contained hexoses in different quantities; pentoses were present only in the products of Bacillus dysenteriae Flexner and Y. Immunity against one of the factors is not associated with immunity against another factor; thus the antiglycemic immunity is independent of the antileukopenic immunity. The leukopenic immunity is on the whole strain specific, although some effects were observed against heterologous strains. The antiglycemic immunity is not strain specific. The hyperglycemic reaction is associated with lowered glycogen content of the liver, lowered galactose tolerance and lowered blood diastase. The leukopenic effect was observed in normal and, with a certain modification, in splenectomized rabbits as well. It was accompanied by characteristic changes in the spleen and bone marrow.

FROM AUTHORS' SUMMARY.

STAPHYLOCOAGULASE. R. CRUIKSHANK, J. Path. & Bact. **45**:295, 1937.

Staphylocoagulase is apparently an enzymic substance, with an action similar to that of thrombin on the plasma of various animal species, including man. Although unrelated to the necrotoxin and alpha lysin of the staphylococcus, staphylocoagulase, like them, is present only in the pathogenic members of the species whether aureus or albus. Coagulase production, because of its constancy and the ease of demonstrating it, is recommended as a test for pathogenicity of the staphylococcus. The part which staphylocoagulase may play in the infective process is discussed.

FROM AUTHOR'S SUMMARY.

CLAUBERG'S TELLURITE MEDIUM IN THE ROUTINE DIAGNOSIS OF CORYNEBACTERIUM DIPHTHERIAE. P. L. SUTHERLAND and J. L. G. IREDALE, J. Path. & Bact. **45**:325, 1937.

Clauberg's tellurite medium is easy to prepare, but care and foresight are required in the preparation and maintenance of stock materials. On this medium the colonies of Corynebacterium diphtheriae are easily recognized by the naked eye after from sixteen to twenty-four hours' incubation, which results in a great saving of time and labor when swabs are examined on a large scale. The medium is superior to Löffler's serum both as regards the number of positive results obtained and in precluding the risk of diagnosing other diphtheroids as C. diphtheriae.

FROM AUTHORS' SUMMARY.

Immunology

CHEMICAL INVESTIGATIONS ON THE ACTIVE PRINCIPLES OF LOCAL SKIN ACTIVITY TO BACTERIAL FILTRATES. G. SHWARTZMAN and S. A. MORELL, J. Exper. Med. **67**:1 and 13, 1938.

Several physicochemical properties of the principles active in the phenomenon of local skin reactivity to bacterial filtrates have been investigated. Ultrafiltration through Zsigmondy filters of graded porosities has shown that the active substances are retained by membranes finer than from 100 to 120 seconds, whereas coarser ones readily permit their passage. The average degree of porosity at this filtration end point indicates a particle size of about 50 to 100 microns. When filtrates were fractionally precipitated with ammonium sulfate, most of the activity of the filtrate was concentrated in the two-thirds saturated portion. Isoelectric properties were studied by means of capillary analysis and cataphoresis. At p_H 3 and below, the substances suspended in the culture filtrates migrated to the cathode; activity in this chamber, however, could not be demonstrated. At p_H 4 and above, reversal to the anode occurred, as the active materials became negatively charged and readily migrated to this chamber. The isoelectric point, therefore, was considered to be between p_H 3 and 4. Preliminary experiments on extraction by adsorption and on p_H stability have been described.

Bacillus coli, meningococcus and Bacillus typhosus have been cultivated in a diffused broth medium by immersing cellophane bags containing saline solution into nutrient broth. An abundant growth occurred inside the bags, while the outside broth remained sterile. Under these conditions, the production of the principles active in local skin reactivity to bacterial filtrates has been consistent. When the culture filtrates so obtained were redialyzed against saline solution, active preparations were obtained which were practically free from nonspecific ingredients.

FROM AUTHORS' SUMMARIES.

TYPE SPECIFIC PROTEINS IN EXTRACTS OF FUSOBACTERIA. C. WEISS and D. G. MERCADO, J. Exper. Med. **67**:49, 1938.

The suggestion by Slanetz and Rettger that fusobacteria may be classified into types on the basis of their cultural and biochemical characteristics was confirmed. Additional data are presented on the following points: the behavior of types I, II and III in relation to several previously untested carbohydrates; the final p_H in 1 per cent dextrose broth; the hydrolysis of starch; the failure to decompose sodium hippurate and citrates; the absence of fibrinolysin and of proteolytic enzymes. By the use of a technic described in the text the anaerobic fusobacteria could be grown in mass cultures. With the methods of Heidelberger and Kendall, proteins that were immunologically type specific were extracted from fusobacteria types I, II and III. This observation, which is in a sense an extension of the work of Lancefield on Streptococcus haemolyticus group A, represents the second instance of a bacterial protein functioning as a determinant of a bacterial type. Preliminary data indicate that group specificity depends on a carbohydrate which is extractable by the methods of Heidelberger and Avery.

FROM AUTHORS' SUMMARY.

ANTIBACTERIAL IMMUNITY TO STAPHYLOCOCCUS PYOGENES. C. LYONS, Brit. J. Exper. Path. **18**:411, 1937.

Staphylococci are encapsulated in the invasive phase. The effective antibacterial antibody is type specific for the capsular antigen.

FROM AUTHOR'S CONCLUSIONS.

INFLUENZA COMPLEMENT FIXATION AND IMMUNIZATION. L. HOYLE and R. W. FAIRBROTHER, Brit. J. Exper. Path. **18**:425 and 430, 1937.

Complement-fixing antigen is produced when the virus of influenza is cultivated on the chorioallantoic membrane of the developing hen's egg. A saline extract of either fresh or dried infected egg membrane forms an excellent antigen for use in the complement fixation reaction. The occurrence of zone phenomena in the influenza complement fixation reaction is described.

The immunogenic factor in virus suspensions is the elementary body. Elementary body suspensions are more easily and satisfactorily prepared from infected mouse lungs than from cultures of the virus on chorioallantoic membranes of developing chick embryos. Elementary body suspensions, inactivated by heat at 57 C. for one-half hour and injected subcutaneously and intraperitoneally into mice, induce a marked degree of immunity. Such suspensions appear to be as effective as living or as formaldehydized virus.

FROM AUTHORS' SUMMARIES.

HAY FEVER: THE SKIN-REACTIVE POTENCY OF PROTEIN AND CARBOHYDRATE FRACTIONS OF TIMOTHY POLLEN. D. HARLEY, Brit. J. Exper. Path. **18**:469, 1937.

Protein and carbohydrate fractions of timothy pollen were prepared. Fraction A (denatured protein of the albumin type) in a dilution of 1:5,000,000 produced specific skin reactions. Fraction A desensitized hay fever serum in passive transfer sites to whole timothy extract. It was concluded that fraction A represents the

idiotoxin of timothy pollen in a denatured but highly active state. The skin-reactive potency of fractions F (denatured protein of the globulin type) and C (carbohydrate plus a small amount of protein) was considerably less than that of fraction A, and it was considered that their activity was probably due to the presence of small amounts of the active albumin. The question of the specificity of the timothy idiotoxin being due to a comparatively small and stable grouping attached to and activated by a protein complex is discussed.

FROM AUTHOR'S SUMMARY.

EXPERIMENTAL OSTEITIS FIBROSA AND THE PARATHYROID GLANDS. W. EGER, Beitr. z. path. Anat. u. z. allg. Path. **100**:19, 1937.

Eger succeeded in reproducing generalized bony changes, most intense in the extremities and closely similar to those observed in human osteitis fibrosa cystica of von Recklinghausen, in young, growing rats by repeated subcutaneous administration of a neutral 1 per cent solution of lead acetate. The lesions in the bones were rather regularly associated with enlargement of the parathyroid glands by adenomatous or nodular hyperplasia, interpreted as secondary to a primary metabolic disturbance.

R. J. LEBOWICH.

CONCERNING EXPERIMENTAL APPENDICITIS. K. HEINEMANN, Beitr. z. path. Anat. u. z. allg. Path. **100**:62, 1937.

Nineteen rabbits were highly sensitized by five subcutaneous injections of normal hog serum at five day intervals, 3 cc. being given at each injection. The appendixes were surgically removed from seven to thirteen days after the last injection. Grossly these organs were the seat of a reaction marked microscopically by mild changes consisting of occasional small areas of necrosis in the germinal centers of the lymph follicles, irregular edema of the serosa and muscularis and leukocytic invasion of the serosa and mesoappendical fat. It is worthy of note that the lumen and mucosa were free from leukocytes, while the mucosal epithelium was intact.

In other series of rabbits sensitization by a mixture of toluene and an inactivated hemolytic serum or hog serum excited a relatively more severe and widespread reaction in the appendix, resembling more closely that in human appendicitis and characterized by a wedge-shaped necrosis of the wall and by edema and leukocytic invasion of all the coats.

The experiments do not permit the drawing of any conclusions as to the origin of human appendicitis.

R. J. LEBOWICH.

THE PRODUCTION OF AGGLUTININS AND THE INTERMEDIATE FAT AND CARBOHYDRATE METABOLISM. S. LAJOS, Ztschr. f. Immunitätsforsch. u. exper. Therap. **90**:261, 1937.

A series of rabbits were given repeated injections of a typhoid vaccine; another series, injections of sodium fluorate, and a third, injections of both. After repeated determinations of the agglutinins, all animals as well as untreated controls were killed and quantitative determinations made of the neutral fat, cholesterol, phosphatides, iodine number, total carbohydrates and glycogen in the liver and of the neutral fat, cholesterol, phosphatides and dextrose in the blood. In all three series there was an increase of fats and a decrease of carbohydrates in the liver and a rise of the phosphatides and a lowering of the other lipoids in the blood, with a drop in dextrose.

I. DAVIDSOHN.

FORMATION OF HEMOLYSINS IN RESPONSE TO MIXTURES OF BLOOD. J. MAGERL, Ztschr. f. Immunitätsforsch. u. exper. Therap. **90**:327, 1937.

Rabbits immunized with mixtures of blood of the horse, beef, hog and sheep responded with a much higher titer of hemolysins for sheep and horse red cells

than did animals given injections of only one kind of blood, while the hemolysins for hogs and beef blood were lower in the animals given injections of the mixtures than in those given the single bloods. Animals given injections of mixtures of horse and sheep bloods showed the same behavior. The same animals given chicken blood after an interval showed a greater increase in animals previously immunized with mixtures than in those immunized with single bloods, and even those that had been originally given injections of only one kind of blood responded to the injection of chicken blood with a higher titer than they did originally. Injection, after an interval, of a yeast suspension caused a depression of the hemolysin titer. The animals given injections of mixed bloods reached the peak of antibody production sooner than the others, and the drop of the antibodies was more gradual.

I. DAVIDSOHN.

SEROLOGIC DIFFERENCES BETWEEN OLD AND NEW WORLD MONKEYS. P. DAHR, Ztschr. f. Immunitätsforsch. u. exper. Therap. **90**:376, 1937.

Neither the A nor the B factor was found in the blood of 35 old world monkeys belonging to fifteen different species. Of 17 new world monkeys belonging to ten different species, 14 had factor B and 2 factor AB. One had none of the factors. The 2 AB monkeys had no anti-A and anti-B agglutinins in their serums. Six lemurs had neither A nor B. The serums of 6 old world monkeys agglutinated the red cells of the new world monkeys but not the cells of the other old world monkeys. The serums of 3 new world monkeys clumped the cells of the old world monkeys except the cells of the species Pithecus. Absorptions suggest the presence of a common receptor in the blood of old world monkeys and the presence of another common receptor in the blood of new world monkeys. Some of the old world monkeys had in their serum anti-A or anti-B agglutinins. None of the examined bloods had the factors M and N.

I. DAVIDSOHN.

BLOOD GROUPS IN BIRDS. P. DAHR and R. ROMMEL, Ztschr. f. Immunitätsforsch. u. exper. Therap. **90**:407, 1937.

The blood cells and serums of 9 birds belonging to the Cathartidae, 5 birds belonging to the Vulturidae, 1 bird belonging to the Falconidae, 18 birds belonging to the Aquilinae and 3 birds belonging to the Butterninae were tested against each other and against human blood cells and serums and with proper absorptions. Serologic relationships were found to exist between the families of vultures and falcons, as well as incompatibilities between these two families and a family of new world vultures. In the serum of some birds group-specific anti-A and anti-B agglutinins were found, but in the red cells of none of them were the properties A, B, M or N found. The agglutination reactions of all these bird species and of human blood are tabulated.

I. DAVIDSOHN.

STAPHYLOCOCCIC INFECTION AND IMMUNITY. J. FORSSMAN, Acta path. et microbiol. Scandinav. **14**:468, 1937.

While Ramon and others hold that pathogenic staphylococci produce a specific toxin and that antistaphylococcic immunity is due to a specific antitoxin, Forssman advances experimental evidence in favor of the view that antistaphylococcic immunity is more broadly anti-infectious in its nature.

IMMUNITY REACTIONS OF A HETEROTRANSPLANTABLE MOUSE CARCINOMA. J. PUTNOKY, Am. J. Cancer **32**:35, 1938.

A biologic difference exists between the mouse and the rat strain of the Ehrlich carcinoma. Rats in which the rat strain had grown and had regressed were resistant to a subsequent transplantation of tumors of rat origin, such as the Jensen rat sarcoma and the Walker rat carcinoma, while rats in which the mouse strain had grown and regressed were much less resistant. This is interpreted as

showing that the cells of the Ehrlich carcinoma, as a result of the continued propagation in the rat, had undergone a biologic alteration. They had adapted themselves to the environment of a rat organism, and the rat-grown strain now represents a separate substrain of the Ehrlich mouse carcinoma.

FROM AUTHOR'S SUMMARY.

RELATION OF THE HYPOPHYSIS TO THE GROWTH OF MALIGNANT TUMORS. H. A. BALL and L. T. SAMUELS, Am. J. Cancer **32**:50, 1938.

The growth of transplanted Walker carcinoma 256 was much slower in hypophysectomized male rats than in paired controls receiving the same amount of food. The change in the growth of this tumor was relatively abrupt after removal of the pituitary and was not a progressive change such as would result from atrophy of some dependent organ. All cells of the tumor must have been equally affected, since the subsequent growth of the tumor was of the same type as during the control period but slower. In previous work Ball and Samuels showed that thyroid secretion is not a factor. The present study indicates that the adrenal cortex can also be excluded as affording an explanation of the effects observed.

FROM AUTHORS' SUMMARY.

INCIDENCE OF METASTASIS OF MALIGNANT TUMORS TO THE ADRENALS. D. A. GLOMSET, Am. J. Cancer **32**:57, 1938.

Of 821 cancers, 445 had metastases to different organs, 34 per cent to the liver, 27 per cent to the lungs, 13 per cent to the adrenals, 6 per cent to the kidneys and 3.7 per cent to the spleen. It is especially noteworthy that, although the kidneys, spleen and adrenals are situated close together and have a similar blood supply, the adrenals have more metastases than either the spleen or the kidneys despite their much smaller size. Of all organically metastasizing tumors, 24.7 per cent metastasized to the adrenals. If those cases in which no description of the adrenal is given are eliminated, this percentage rises to 25.9 per cent. This makes the incidence in the adrenals per unit of weight higher than that in the thyroid, lung, spleen, liver or kidney. The high incidence of metastasis to the adrenals from tumors that have their primary site in the lung and breast or in a malignant melanoblastoma leads one to surmise that most adrenal metastases are blood borne. These figures are in accord with those of Willis, which show that adrenal metastases are most commonly bilateral but that when they are unilateral they are more prevalent in left than in right adrenal glands. Tumors which metastasize to the adrenals only occasionally are those of the gastrointestinal tract and the pelvic organs.

FROM AUTHOR'S CONCLUSIONS.

THE CANINE PROSTATE IN RELATION TO NORMAL AND ABNORMAL TESTICULAR CHANGES. S. ZUCKERMAN and T. MCKEOWN, J. Path. & Bact. **46**:7, 1938.

Examination of 243 dogs of all ages and breeds showed that the size of the prostate is variable in each age group. After about the eighth year of life, the prostate may either undergo involution or continue to enlarge, the two changes being equally common. Where all the specimens are considered together by the method of partial correlation coefficients, both age and body weight appear to influence the size of the organ. Similar analysis of 47 prostates of fox terriers failed to show any correlation with either age or body weight. Enlargement is due either to glandular hyperplasia or, more rarely, to squamous metaplasia of the cylindric prostatic epithelium, which results from spontaneous estrogenic stimulation. Involution is shown by regression of the glandular acini and by reduction in size of the whole organ. The most pronounced involution was observed in 2 castrated dogs. Testicular tumors were present in 35 dogs. Three were neoplasms of the interestitial cells, and the prostates of these animals were normal, 1 being involuted. Seventeen were seminomas, and the corresponding prostates were also normal, 2 being involuted. The remaining 15 were classified under the

term "adenocarcinoma" and were homologous with the mixed epithelial testicular tumors of human pathology, from which they differed in being less differentiated and polymorphic. Nine of the corresponding prostates were normal, 4 being involuted. Carcinoma had occurred in the tenth prostate, and the remaining 5 had undergone squamous metaplasia, presumably as a result of stimulation by estrogenic hormone elaborated by the tumors. The estrogenic potency of such adenocarcinomas is related to the fact that Sertoli cells take part in their formation and that these cells are homologous with the granulosa cells of the ovarian follicles.

FROM AUTHORS' SUMMARY.

Tumors

SPECIFIC PRECIPITATING IMMUNE SERUMS AGAINST CANCER. A. TRAWINSKI, Ztschr. f. Immunitätsforsch. u. exper. Therap. **90**:85, 1937.

Fresh cancer tissue was dried, pulverized, suspended in physiologic solution of sodium chloride, extracted in the ice box and then used for immunization of rabbits. The antigen for the precipitation test was prepared from the aforementioned extract by filtration, inactivation and dilution up to 1:20,000. Preservatives were not used. Antigenic extracts were also prepared from benign tumors and from normal organs. The ring test was employed for the estimation of precipitin. It emphasizes the need of great care and of experience in reading the test. The antiserums reacted only with the homologous antigen except an antiserum against a cancer of the liver, which reacted also with a cancer of the stomach. They did not react with extracts of normal organs, nor did the antiserums for normal organs react with cancer extracts. The cancer extracts did not react with serums of pregnant women and only with 1 of 78 syphilitic serums.

I. DAVIDSOHN.

EXPERIMENTALLY INDUCED EWING SARCOMA IN THE RABBIT. O. SCHÜRCH and E. UEHLINGER, Ztschr. f. Krebsforsch. **45**:240, 1937.

Rabbits were given intraosseal injections of petrolatum containing from 2 to 5 micrograms of radium or mesothorium. Of 18 rabbits that lived eighteen months later, sarcoma had developed in 9. In 3 there were intraosteal and periosteal spindle cell sarcomas; in 1, an extraosseal fibrosarcoma; in 4, very undifferentiated osteogenic sarcomas, and in 1, a typical sarcoma of the Ewing type. This animal died after thirty-six and a half months with generalized, blood-borne sarcomatosis. It appears that irradiation is quite nonspecific as regards the type of tumor produced.

H. E. EGGERS.

MEASUREMENTS OF NUCLEI IN CERVICAL EPITHELIUM, CERVICAL CARCINOMA AND UTERINE MUCOSA. W. STRODTBECK, Ztschr. f. Krebsforsch. **45**:268, 1937.

In cervical stratified squamous epithelium the ratio of the volume of the nucleus of the cell of the basal layer to that of the cell of the stratum spinosum is 1:2. The absolute volumes correspond to those found by other observers for skin, epithelium of the tongue and tonsillar epithelium. In cervical carcinoma the nuclear volume was found to be that of the stratum spinosum. Evidences of rhythmic growth were observed in cervical carcinomas and also in inflamed cervical erosions. In 3 cases of carcinoma of the fundus no variations in nuclear dimensions from those of normal endometrium were found. In view of this fact and of the correspondence in nuclear volume between cervical carcinoma and normal stratum spinosum, Strodtbeck is inclined to doubt the significance of Ehrich's reported observation of doubled nuclear volumes in cancer.

H. E. EGGERS.

ESTIMATING THE TIME OF DEATH FROM THE APPEARANCE AND THE FINDINGS ON EXTERNAL EXAMINATION OF THE BODY. B. MUELLER, München. med. Wchnschr. **84**:1021, 1937.

Any physician may be called to inspect a body and express an opinion as to the time that has elapsed since death. While it cannot be expected that every physician should be conversant with all the special methods that permit accurate determination of the interval since death, it is advisable that every physician should familiarize himself with the important observations that can be made only at the first inspection of the body and that he record them. Somebody else may later interpret his observations. These observations are are follows: During the first twenty-four hours after death the pupil may be deformed by pressure on the eyeball; later this is not possible. It is important to note whether the eyelids are found open or closed. If they are open, drying up and clouding of the cornea set in as early as within one and three-quarters of an hour. When the eyes are closed, the turbidity of the cornea does not set in until twenty-four hours after death. The iris of a drowned person with light eyes turns brown after twenty-four hours. The length of the beard should be measured. It is commonly assumed that the hair of the face grows about 0.5 mm. in twenty-four hours. There are wide variations in this respect. The reported growth of the beard after death is due to drying out of the skin and not to true growth. The temperature of the skin, particularly of that on the covered parts of the body, should be estimated, and at the same time the outside temperature should be recorded. Lividities appear first on the sides of the neck, later in the other regions. In the first four to six hours, pressure on the livores will remove them completely; later hemolyzed serum imbibes the tissues and pressure will cause only some fading, not complete disappearance. After twenty-four hours, pressure causes no change. In case of strangulation, one can find subcutaneous hemorrhages which are not affected by pressure. A low temperature may cause contraction of the smooth muscles of the skin even in the cadaver and so produce the appearance of goose skin. After death relaxation of the muscles of the face occurs, and the features become expressionless. Rarely rigor mortis occurs immediately after death, causing fixation of the position which was present at the time of death. As a rule rigidity sets in from one to two hours after death in the jaw and then progresses downward. It disappears in the reverse order of its appearance and is usually gone after from forty-eight to sixty hours. If it is present in all muscles, one may conclude that death has occurred not less than from five to eight hours ago. If rigidity is forcibly dispelled, it recurs, but only if it is dispelled during the first eight hours. Loosened later, it does not recur. Loosening should be tried and the result recorded. If rigidity has not yet set in, one should look for evidences of mechanical irritability of muscles by percussion. It may be detected from one to two hours after death. Flies lay eggs in the angles of the mouth and eyes soon after death. The presence of maggots indicates that the body was in its location from eight to twenty-four hours. It takes from ten to twelve days for pupae to develop and from thirteen to fourteen more days for pupa shells to form. The temperature of the rectum should be recorded. In bodies of persons who had high fever before death, further elevations were occasionally observed after death. In general, the drop of rectal temperature amounted to from 0.25 to 1.5 C. per hour, with variations depending on the nutrition, the clothing and the outside temperature.

I. DAVIDSOHN.

Microbiology and Parasitology

THE PATHOLOGIC-ANATOMIC CHANGES INDUCED BY ELECTRICAL CURRENTS. F. PIETRUSKY, Deutsche Ztschr. f. d. ges. gerichtl. Med. **29**:135, 1938.

The cause of death following electrical shock is not always evident and must at times be ascribed to functional disturbances. In many instances it is difficult to ascertain whether death is due to coexisting disease or to secondary trauma.

There is uniformity of the pathologic-anatomic alterations on the living body so far as they are at times thermal and at other times electromechanical. It is known that electrical currents are used for committing murder and suicide and that the cutaneous lesions produced by current can be instituted post mortem. Since the evidence of electrical action often is lacking on the skin, one must rely on changes in the clothing and on other circumstances for proof of such action. A detailed discussion of the lesions induced by electricity in the various organs is included. Caution is advocated in designating a burn as electrical when other alterations in the body corroborating this are lacking. The necessity for attributing death to functional changes is stressed.

GEORGE J. RUKSTINAT.

ESTIMATION OF THE AGE OF NEWBORN AND PREMATURE INFANTS FROM CHARRED BONES. G. SCHRÄDER, Deutsche Ztschr. f. d. ges. gerichtl. Med. **29**:152, 1938.

The research material consisted of the bodies of 16 children close to maturity, weighing from 1,340 to 3,480 Gm. and measuring from 34 to 56 cm. in length. One half of each body was burned; the other half was macerated. In this manner, comparable material was acquired for measurement. By this method, it was found that burned bones became shorter than those that were macerated. The bones also shrank in other dimensions, so that estimates of the age of the child might be as much as one to two months in error. Thus it was found that the macerated bones of a child 45 cm. long were slightly larger than those of a child 50 cm. long which had been burned.

GEORGE J. RUKSTINAT.

THE ESTIMATION OF THE TIME OF DEATH FROM THE CARDIAC PLASMA CONTENT. A. PONSOLD, Deutsche Ztschr. f. d. ges. gerichtl. Med. **29**:163, 1938.

Soon after the heart stops beating, there is a separation of the blood into cells and plasma. The latter accumulates in the region of the exits of the chambers and is pushed out by the contracting force of cardiac rigor. This shift of plasma is followed by condensation of blood in the left ventricle and can be measured by the hematocrit. In this way, from a percental estimation of the plasma content of the heart, the duration of death may be determined up to twenty-four hours. It is important that there be no marked premortem change in the plasma, no leaking of blood from the body and no weakening of the heart muscle from disease. It is also important that the body lie on its back between the time of death and the estimation of the percental content of plasma. The relation between the plasma content of the heart's blood and the time after death is as follows:

Plasma Content, per Cent	Hours After Death
40	6
30	12
20	18

GEORGE J. RUKSTINAT.

Society Transactions

BUFFALO PATHOLOGICAL SOCIETY

ERNEST WITEBSKY, *President*

June 11, 1938

SAMUEL SANES, *Secretary*

A STUDY OF THE INTESTINAL TRACT IN SO-CALLED HEMATOGENOUS PERITONITIS IN CHILDREN. KORNEL TERPLAN and ELIZABETH HYDE.

From a series of about 28 cases of so-called hematogenous peritonitis we selected for this presentation 7 which have been studied fairly completely. As controls for histologic and bacterioscopic analysis, we used a few cases of diffuse peritonitis following rupture of a phlegmonous appendix or intussusception by Meckel's diverticulum. A general summary of the findings follows.

In the lumen of the lower part of the ileum there is some inflammatory exudate. The surface epithelium is intact except for small superficial erosions. The underlying mucosa shows rather moderate inflammation and a few cocci. The most marked lesions occur in and around the lymph follicles, which contain in their germinal centers small abscess-like formations; the sinusoids and lymph spaces contain gram-positive cocci, phagocytosed and free. The submucosa shows only the mildest changes and the muscle layers practically none. The serosa and subserosa are markedly inflamed and contain many bacteria and much fibrin. This inflammation decreases in severity and in the number of bacteria as the outer muscle layer is approached and does not extend into the muscle layers. The general picture, microscopically, in all 7 cases presents the same features: marked inflammation and bacterial invasion of the serosa and subserosa, inflammatory exudation from the serosa; little if any change and few or no bacteria in the muscle layers and submucosa. The mucosa shows changes, it is true, and bacterial invasion by cocci, but the lesions are no more severe and are of the same order as those seen in controls in which there had been stasis and signs of ileus, which may be responsible for the changes in the condition under discussion. It is notable that in all bacterioscopic studies only cocci, and apparently streptococci, as far as morphologic study can determine, were seen in the lesions. Certainly no bacilli were seen except within the lumen of the intestine. The studies were made on sections of many different levels of jejunum, ileum and colon, stained with hematoxylin and eosin, with methylene blue and with Gram's stain.

These findings do not support the theory that this type of peritonitis, so commonly observed in children, is brought about by extension of streptococci from the lumen of the intestine, through the wall, into the serosa and into the peritoneal cavity.

In every case in this series a bacteriologic examination was made, but in this presentation we wished to discuss only the tissue changes in the intestinal tract and their significance in relation to the still debated question of the pathogenesis of primary peritonitis.

ACTION OF SULFANILAMIDE ON FIBRINOLYSIN AND HEMOLYSIN PRODUCED BY STREPTOCOCCI IN VIVO. ERWIN NETER.

The therapeutic effectiveness of sulfanilamide and related compounds in the treatment of infections due to hemolytic streptococci is established beyond doubt, but the mode of action of this drug is still in question. It has been shown that

sulfanilamide exerts a certain degree of bacteriostatic activity toward hemolytic streptococci in vitro. Furthermore, it is reported that prontosil ([1] the hydrochloride of 4-sulfamido-2′,4′-diaminoazobenzene, or [2] the disodium salt of 4-sulfamidophenyl-2′-azo-7′-acetylamino-1′-hydroxynaphthalene-3′,6′-disulfonic acid) may counteract the action of streptococcic hemolysin in vitro (Meyer, F.: *Quart. Bull., Sea View Hosp.* **2**:148, 1937).

Experiments are here reported dealing with the action of sulfanilamide on the hemolysin and fibrinolysin produced by streptococci in vivo. As previously reported (Neter, E.: *Proc. Soc. Exper. Biol. & Med.* **34**:735, 1936. Neter, E., and Uhry, E.: ARCH. PATH. **24**:405, 1937), peritoneal washings of mice infected with hemolytic streptococci may exhibit fibrinolytic as well as hemolytic activity. The action of sulfanilamide on such fibrinolytic and hemolytic exudates was tested, and the following results were obtained: Sulfanilamide in concentrations ranging from 0.8 to 0.008 per cent failed to inhibit the fibrinolytic activity of such exudates. This result parallels the observations of Madison and Snow (*Proc. Soc. Exper. Biol. & Med.* **36**:592, 1937) and of Huntington Jr. (ibid. **38**:328, 1938) on the failure of sulfanilamide to inhibit fibrinolysin produced by hemolytic streptococci in vitro. On the other hand, sulfanilamide in the concentration of 0.8 per cent inhibited the hemolytic activity of peritoneal washings of mice infected with hemolytic streptococci; smaller concentrations, such as 0.08 per cent and less, however, only delayed hemolysis or proved to be ineffective.

The experiments reported show that sulfanilamide fails to inhibit the action of fibrinolysin produced by hemolytic streptococci in vivo. Its effect on the hemolysin produced by these micro-organisms in vivo is demonstrable with concentrations above those usually encountered in patients treated with this drug. It may be concluded, therefore, that the action of sulfanilamide on the hemolysin and fibrinolysin produced by hemolytic streptococci does not play a major part in its therapeutic effectiveness in streptococcic infections of animals and man.

A CONFIRMATION TEST FOR THE SERODIAGNOSIS OF SYPHILIS. ERNEST WITEBSKY.

It is often difficult to decide whether a positive reaction obtained by one of the methods available for the serodiagnosis of syphilis is specific or not. The "confirmation test" may decide the issue. This test is made as follows: The serum under investigation is mixed with a certain amount of diluted extract so that flocculation is obtained. The floccules are washed and suspended in saline solution at 56 C. After incubation for from twenty to thirty minutes, the test tube containing the floccules is centrifuged as quickly as possible. The supernatant fluid contains the antibodies as isolated from the syphilitic blood serum. This antibody solution reacts in the same way as does the nonabsorbed native serum and may be used in complement fixation as well as in flocculation tests. Nonspecific reactions due to lability of the serum are not caused by antibodies, and the supernatant fluid in such cases is entirely negative.

Experiments were carried out with serums that reacted positively simultaneously in the serodiagnosis of syphilis, gonorrhea and tuberculosis or in the serodiagnosis of any two of them. Those serums were absorbed with the respective antigens. It could be demonstrated that it is possible to separate again the antibodies from the antigens. This proves that positive results obtained in those different tests are due to independent antibody functions and are produced by different causative agents.

Serodiagnosis of syphilis in rabbits is usually considered to be impractical because of the rather frequent nonspecific positive results obtained. The confirmation test allows one to decide whether or not antibodies are present in the rabbit serum, or, in other words, whether one is dealing with a specific or a nonspecific reaction. Therefore, this method may be applied in cases in which there is no certainty as to the specificity of a positive reaction. It proves further that the blood change occurring in syphilis is not a simple "lability" reaction but is caused by the formation of antibodies.

SO-CALLED FELTY'S SYNDROME: REPORT OF CASE WITH AUTOPSY. S. SANES, L. M. LOCKIE and S. L. VAUGHAN.

The clinical findings which constitute Felty's syndrome were first described in 1924. These findings comprise essentially: chronic recurring arthritis, splenomegaly, marked leukopenia and granulopenia. Other less constant features include loss of weight, secondary anemia, fever, hepatomegaly, enlargement of lymph nodes, pigmentation of the skin and eosinophilia. We present the postmortem observations in a case which must be considered under the heading of Felty's syndrome.

The patient was a 53 year old unmarried woman. For twenty years she had suffered from chronic recurring arthritis, diagnosed as atrophic arthritis of the chronic infectious type. During three years before death, she lost 26 pounds (11.8 Kg.). On admission to a hospital she had no complaints except "arthritis." The submaxillary glands appeared swollen. The axillary and inguinal lymph nodes were pea sized. The liver and spleen were palpable.

The red blood cell count was 2,540,000 per cubic millimeter, with hemoglobin 50 per cent. The white blood cell count was 1,900 per cubic millimeter. A blood smear showed: filaments, 12 per cent; bands, 3 per cent; eosinophils, 6 per cent; lymphocytes, 54 per cent; monocytes, 19 per cent; undetermined, 3 per cent; reticulocytes, less than 1 per cent; platelets, normal.

Only the pertinent findings at autopsy will be mentioned. Grossly, marked arthritis was found in the joints of the hands and feet and in the sternoclavicular joint. The spleen weighed 380 Gm. The liver weighed 1,800 Gm.; it reached to the iliac crest. The abdominal lymph nodes were moderately enlarged. There was generalized anemia.

Histologic examination of the sternoclavicular joint disclosed rather typical evidences of chronic proliferative arthritis. In the spleen the follicles were not large. The trabeculae were not prominent. There was dilatation of sinuses, with sinus endothelial hyperplasia. Outstanding in the sinuses were many plasma cells and macrophages. A number of the latter were multinucleated and phagocytic. Peroxidase-positive cells were also found. The intersinusoidal cords were thickened. Stains for bacteria and iron gave negative results. Fatty changes were present in the central zones of the liver. An abdominal lymph node showed a chronic nonspecific inflammatory hyperplasia.

In a differential smear of the sternal bone marrow the following types of cells were present in the percentages given: myelocytes, 8; juveniles, 14; bands, 17; filaments, 2; eosinophils, 1; basophils, 1; lymphocytes, 3; monocytes, 3; plasma cells, 1; reticulum cells, 1; lymphoid cells, 30; normoblasts, 5, and megaloblasts, 2.

This report is of interest because up to 1938 only 4 cases of Felty's syndrome with autopsy were reported in the American literature (Price and Schoenfeld, 1934; Williams, 1936; Singer and Levy, 1936). On the Continent similar cases are described as instances of Chauffard's disease.

Obituaries

EARL BALDWIN McKINLEY, M.D.

1894-1938

Earl Baldwin McKinley, dean of George Washington University Medical School and president of the American Association of Pathologists and Bacteriologists, was among the passengers on the Hawaii Clipper lost on the Pacific Ocean, July 28, 1938, in the course of the Clipper's last flight.

The mission on which Dr. McKinley was engaged was typical of a life characterized by driving energy and courage. His aerial flight was no mere holiday excursion but a carefully planned project devoted to the advancement of scientific knowledge. With Dr. Fred C. Meier, senior scientist in the Extension Service of the United States Department of Agriculture, McKinley was engaged in a study of bacteria, pollen and other microscopic life in the upper air levels. Their investigation was under the auspices of the Committee on Aerobiology of the National Research Council, of which Meier was chairman and McKinley an active member. Through years of developmental research Meier had become proficient in the collection of bacteria through airplane flights, and the time seemed ripe for a study of those long drifts of minute life known to occur in the moving air far above the earth, which as yet have been little studied by exact methods. McKinley embarked on the flight with a double object. He was intensely interested in the possibilities for the study of the bacteria of the air and as a co-worker with Meier in this problem shared in operating the collecting apparatus throughout the flight. But the hard work of the trip was not to end with completion of the trip across the ocean. With the cooperation of former close medical associates in Manila, McKinley had meticulously organized a survey of a large number of Filipinos in an environment of endemic leprosy, to test the efficacy of certain antigenic substances, comparable to tuberculin, in the diagnosis of leprotic infection. These substances were prepared partly in his own research and partly in the course of investigations at the Henry Phipps Institute in Philadelphia and Yale University, investigations sponsored by the Leonard Wood Memorial for the Eradication of Leprosy, with which McKinley had long been intimately connected. A large survey was to be completed in a few days of intensive effort, so that he could return promptly to Washington. A thorough groundwork had been laid by McKinley and his

associates in a previous study on 5,000 residents in the same region. Without counting the cost in strength, far less the danger, he embarked on this formidable schedule with everything in his own power well arranged and with a buoyancy of spirit that all his friends will remember as characteristic.

Dr. McKinley was born in Emporia, Kan., Sept. 28, 1894. Influenced by an instructor in history while he was in high school at Newton,

EARL BALDWIN McKINLEY, M.D.
1894-1938

Kan., he selected the University of Michigan for his university course. He graduated in 1916. A year later he was married to a college mate, Leola Edna Royce, of Sault Sainte Marie, Mich. Two children, Elisbeth Janet and Royce Baldwin McKinley, came from this union. In 1917 McKinley went overseas as a lieutenant in the Rainbow Division of the American Expeditionary Force, to serve as an intelligence officer. At the close of the war he returned to Michigan to commence the study of medicine.

He graduated in medicine at the University of Michigan in 1922, and from this year forward his life was a series of positions of increasing responsibility. As a medical student, under the influence of Vaughan and Novy, he had acquired a strong taste for bacteriology and for physiological chemistry, and for three years before graduation he was an instructor in these subjects at the University of Michigan. In 1922 he accepted a call to Baylor University, Texas, as assistant professor of bacteriology, and a year later he was made chairman of the department.

During his vacation periods in Dallas, Texas, he began the practice of pediatrics. This practical experience in medicine gave him an insight into the current difficult medical problems not ordinarily possessed by men with a purely academic background. But medical practice was not to his taste, and in the meantime a steadily growing interest in the fundamentals of his science, probably coupled with the realization that increasing responsibility would soon make further uninterrupted study impossible, led him to apply in 1924 for a fellowship of the National Research Council for study abroad. Successful in the application, he chose a year with Bordet at the Pasteur Institute, Brussels, Belgium. Under Bordet's direction he investigated the lytic and other toxic principles of serum and began studies on encephalitis, a field in which he maintained interest thenceforth.

His work attracted the attention of Frederick Gay, who in 1925 invited him to enter the Department of Bacteriology of the College of Physicians and Surgeons of Columbia University as assistant professor. He was promoted to the position of associate professor the following year. After two years the urge for wider experience led him to resign from his academic position to become a field director of the International Health Division of the Rockefeller Foundation in the Philippines. It was here, if not earlier, that his capacity for organization became apparent. Maintaining headquarters at the Bureau of Science in Manila, he took an active part in promoting health activities. He lectured at the University of the Philippines and was influential in the development of the School of Hygiene and Public Health at Manila. A lasting interest in leprosy began, and it was his concern with this field, as well as his rich and varied experience in other problems of the tropics, that brought about his interest in tropical medicine, dominant thenceforward. His membership in and promotion of the organization and scientific development of the Society of Tropical Medicine, the Academy of Tropical Medicine and the American Foundation for Tropical Medicine testify to the strength of this interest, which was also in turn responsible for his most productive scientific work.

In 1928 he was called back to Columbia University with the rank of professor. His familiarity with tropical medicine and his talent for

administration made him an ideal choice for the directorship of the School of Tropical Medicine of the University of Puerto Rico, which had been organized in 1926 and maintained under the auspices of Columbia University. During the three busy years of his administration the hospital of this school was built and in spite of the routine detail involved he found time for his best known research, with Malcolm Soule of Michigan: the isolation of a micro-organism believed by these investigators to be the specific cause of leprosy.

In 1931 he was invited to accept the deanship of the Medical School of George Washington University, with additional responsibilities and opportunities as professor of bacteriology and director of medical research. Attracted by the prospect of promoting medical science in the nation's capital, he accepted. In Washington his cares were heavily multiplied. Brought to this university specifically to develop the medical school, he taught classes, steadily promoted research and inaugurated lectureships, while faithfully discharging the endless duties of school and hospital administration. The necessities of organization in the medical school and hospital inevitably led to intimate concern with the medical problems of Washington. The controversial question of medical care excited his sincere and serious interest, and the search for a solution equitable for physician and patient alike in Washington absorbed much of his time and strength. During the Washington period he gave service to many scientific organizations. Aside from those devoted to tropical medicine and the several Washington societies in which he was active, the American Association for the Advancement of Science and the Leonard Wood Memorial for the Eradication of Leprosy consumed the greatest part of his attention. He was a member of the executive committee of the American Association for the Advancement of Science for four years, and the present highly successful symposiums in the meetings of this association are largely the result of his initiation and effort. Leprosy was the chief field of his scientific investigation in recent years, and as secretary of the medical advisory board of the Leonard Wood Memorial for the Eradication of Leprosy, member of the first commission of this organization to study scientific problems and constant advisor to the president of the board, he promoted leprosy investigation in many ways. He was also a member of the executive committee of the Division of Medical Sciences of the National Research Council. His most recent responsibility and honor came in May 1938, when he was elected president of the American Association of Pathologists and Bacteriologists for the ensuing year.

His best known researches were in the fields of leprosy, filterable viruses, bacteriophages and the geography of disease. With Soule, as previously noted, he had isolated a unique, slow growing micro-organism from leprotic nodules, now recognized as one of the strongest con-

tenders among the numerous acid-fast organisms isolated from leprosy for the distinction of being the true cause of this mysterious disease. While with the International Health Division and the Bureau of Science in Manila, he published a comprehensive review, entitled "Filterable Virus and Rickettsia Diseases." He was the author of a lengthy review of the same general subject and the problem of the bacteriophage in Gay's "Agents of Disease and Host Resistance." In recent years he displayed a growing interest in statistics on mortality. He published a "Geography of Disease" in 1935 and immediately thereafter began a similar "Cartography," in which the world distribution of disease was to be manifest in a series of maps.

This immense amount of work was made possible only by an exceptionally strong physique and the capacity for long hours of action. Indeed, much of McKinley's work was done in his home in the late hours of the night. Sociable, fun-loving, generous and hospitable, he entertained guests constantly. Few knew that he returned to his desk for prolonged labor after they had retired. His eminent position was indeed due to two factors: a thorough knowledge of medical research, thus maintained, and an unusually extensive acquaintance with the scientific men of the country.

He will be remembered by his many friends for his heartiness and cheery comradeship. All who knew him even slightly will recall the enthusiasm and drive that formed the keynote of his life.

ESMOND R. LONG.

Book Reviews

Dictionnaire des bactéries pathogènes pour l'homme, les animaux et les plantes. By Paul Hauduroy and others. Price, 140 francs. 597 pp., with 5 tables. Paris: Masson & Cie., 1937.

It is true, as the preface informs the reader, that many who work in the field of human bacteriology know relatively little about the species of bacteria pathogenic for lower animals and plants, that the veterinary bacteriologist suffers from similar unfamiliarities and that the phytobacteriologist is often unaware of the descriptions of species encountered by his colleagues in the other fields. To pool the separate sources of information, the authors have united in the form of a simple dictionary summaries of knowledge of the bacteria pathogenic for man, lower animals and plants. The material on the bacteria that infect man was contributed by Paul Hauduroy and G. Ehringer, that on the bacteria of animals by A. Urbain and G. Guillot and that on the bacteria of plants by J. Magrou.

The combined compilations of these authors are presented in the form of an alphabetical classification of 650 species of pathogenic bacteria. The orders, families and genera, and in each genus the species, are listed alphabetically. Any system of alphabetical arrangement has its absurdities, but the authors decided wisely that this was better than an alphabetical classification based on the species names. As a matter of fact, the excellent index is itself an alphabetical listing of the names of species.

The work is not a treatise on bacteriology and does not contain descriptions of methods, mediums or diagnostic procedures. Under the name of each organism is given a reference to the first published description of it, a list of synonyms and brief summaries of information concerning its forms, cultural characteristics, biochemical activities and biologic properties, such as pathogencity, vitality and resistance, and its relation to host and habitat. These terse descriptions contain numerous statements of facts not given in the original articles cited. These statements, however, are not supported by references to later publications. For example, the paragraphs under the heading "Proteus vulgaris" contain comments on the Weil-Félix reaction and on the isolation of this organism from patients suffering with typhus fever but do not refer the reader to the sources of the information. The same may be said of other descriptions. Obviously, completely documented articles as condensed as these are would contain nearly as many references as statements. As this would defeat the purposes of convenience for which the book was compiled, the bibliography was intentionally reduced to a minimum.

The nomenclature used is erroneously called that of the Society of American Bacteriologists. By this the authors mean Bergey's "Manual of Determinative Bacteriology," to which they acknowledge great indebtedness. While the descriptions are often translations or paraphrases of the language in Bergey's Manual, they are also amplified and enriched by additional information concerning cultural characteristics, serologic reactions and pathogenic properties.

The authors do not accept Bergey's nomenclature but use it for convenience. They would have preferred adopting nomenclatures for the genera created by other scientists, particularly A. R. Prevot. These nomenclatures, however, are incomplete, and their adoption would have made it impossible to compose a homogeneous dictionary. The chaotic state of bacteriologic nomenclature is so disturbing that the authors were forced, as is any one who attempts to make lists of bacteria, to devote a special section to a discussion of this recurrent topic. They emphasize the fact that, paradoxic as it may seem, there are no officially adopted rules determining precisely the principles of nomenclature of the bacteria and that there is no nomenclature which can be adopted universally. A bacteri-

ologist is free today to give any name he chooses to a known bacterium or to a newly discovered species. The long lists of synonyms are striking evidence of the freedom, bordering on nomenclatural license, with which bacteriologists repeatedly rename previously named species. Often, however, ignorance of previous work or failure to recognize a species by the description of it accounts for the rediscoveries and for the multiplication of new names.

To assist in bringing some order into this chaos, the authors call attention to mechanisms for establishing names, and they review the attempts to secure recognition of systematic rules. For this purpose they reprint appropriate sections of the International Rules of Botanical Nomenclature, using the French version of Hochreutiner, published in 1935. No doubt the majority of students of the subject would agree that the rules of international conventions of botanists should be used for naming bacteria, but it must be admitted that there are a number of bacteriologists who still think that these rules are not entirely suited for determining bacterial nomenclature. This book helps to bring these matters to the fore again but does not answer the questions.

Although workers in this country have been ingenious and fertile in producing unnecessary and incongruous names for bacteria, it is gratifying to find that Hauduroy and his associates not only render a general tribute to American systematic bacteriologists but by adopting the Bergey nomenclature "provisionally" and by reproducing much of the material in the Manual also pay a tribute of usage.

This dictionary is not provided with keys to genera and species. Therefore to locate the description of an organism the searcher must have at hand some name for it, applying the well known principle that the use of a dictionary depends on an adumbration of spelling. With a slight clue, however, the species can be tracked down fairly readily by use of the index and the alphabetically arranged descriptions.

The book contains several appendixes. One is the list of bacteria belonging to the genus Salmonella as defined by the subcommittee of the Committee on Nomenclature of the International Society of Microbiology. Following these lists there is a series of useful tables of serologic and fermentation reactions of the Salmonella group and of typhoid and dysentery bacilli and a summary of Bergey's classification of streptococci. Tables I to V at the end are large differential diagnostic tabulations of the characteristics of organisms of the following genera: Aerobacter, Alcaligenes, Eberthella, Escherichia, Proteus, Salmonella and Shigella.

The dictionary contains a brief mention of the actinomycetes but does not contain descriptions of yeasts, molds and fungi. A separate part of the index is composed of an alphabetical table of the names of the plants cited in the text.

I have used this book to some extent as an aid in the identification of bacteria. It gives some useful supplementation of knowledge of known species but is not conclusive in instances of debatable or uncertain information, as for example in the identification of spore-bearing anaerobes and of bacteroides. My actual experience with the book is still insufficient to be a test of its usefulness in dealing with veterinary or plant bacteriology. Its possibilities, however, are recognizable by inspection of the contents, and it should prove to be of considerable practical value to diagnostic and systematic bacteriologists.

Books Received

A B C OF THE VITAMINS: A SURVEY IN CHARTS. Jennie Gregory, M.S. Foreword by Walter H. Eddy, Professor of Physiological Chemistry, Teachers College, Columbia University. Cloth. Pp. 93. Price $3. Baltimore: Williams & Wilkins Company, 1938.

REPORT OF THE PENROSE RESEARCH LABORATORY, FORMERLY LABORATORY AND MUSEUM OF COMPARATIVE PATHOLOGY OF THE ZOOLOGICAL SOCIETY OF PHILADELPHIA, IN CONJUNCTION WITH THE SIXTY-SIXTH ANNUAL REPORT OF THE SOCIETY. Herbert Fox, M.D., Pathologist. Paper. Pp. 67. 1938.

THE HARVEY LECTURES. DELIVERED UNDER THE AUSPICES OF THE HARVEY SOCIETY OF NEW YORK, 1937-1938. UNDER THE PATRONAGE OF THE NEW YORK ACADEMY OF MEDICINE. Dr. Selig Hecht, Dr. Einar Lundsgaard, Dr. Cecil K. Drinker, Dr. John P. Peters, Dr. Philip Bard, Dr. Wendell M. Stanley, Dr. F. C. Koch, Dr. Harry Goldblatt. Series XXXIII. Cloth. Pp. 275, with illustrations. Price $4. Baltimore: Williams & Wilkins Company, 1938.

PATHOLOGICAL TECHNIQUE. Frank Burr Mallory, A.M., M.D., S.D., Consulting Pathologist to the Boston City Hospital, Boston, Mass. Cloth. Pp. 434, with 14 illustrations. Price $4.50. Philadelphia: W. B. Saunders Company, 1938.

AGOSTINO BASSI IN THE HISTORY OF MEDICAL THOUGHT—A. BASSI AND L. PASTEUR—THE CONTAGIUM VIVUM THEORY THROUGHOUT THE CENTURIES: ASPECTS AND CONSIDERATIONS. Giovanni P. Arcieri, M.D. Paper read before the medical staff of the Parkway Hospital, New York, at its meeting of Feb. 23, 1938. Preface by Prof. Cesare Frugoni. Paper. Pp. 30, with portraits. New York City: The Vigo Press, 1938.

CORRECTION

In the article by Douglas Symmers entitled "Giant Follicular Lymphadenopathy With or Without Splenomegaly," in the September issue (ARCH. PATH. **26**:603, 1938), the second sentence in the first paragraph under "Comment" near the bottom of page 622 should have read:

"At Bellevue Hospital there have been encountered at necropsies 17 cases of dermatitis exfoliativa; in 4 of these cases, in which the necropsies were performed under the auspices of the Office of the Chief Medical Examiner, the condition was associated with poisoning by arsphenamine."

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